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FURTHER OBSERVATIONS SHOWING THAT EPINEPHRIN FROM THE ADRENALS IS NOT INDISPENSABLE

G. N. STEWART AND J. M. ROGOFF

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Received for publication March 6, 1919

We showed in a previous paper (1) that cats live indefinitely in good health after excision of one adrenal and section of the nerves of the other, an operation which either abolishes the output of epinephrin from that adrenal or reduces it to a small fraction of the normal. It is well known that a certain proportion of rabbits survive double adrenalectomy, in our experience something like 20 per cent (2). Whether this is due in all cases to the presence of accessory adrenals or not, it is clear that in rabbits also the epinephrin secretion of the adrenals is not indispensable since accessory adrenals consist only of cortical tissue. In this investigation we have extended the observations to dogs and monkeys, with the same result; neither the length of life nor the health of the animals could be shown to be in any way affected by removal of one adrenal and section of the nerves of the other. Some protocols with samples of tracings used in assaying the epinephrin in the adrenal vein blood at the time the animals were sacrificed are reproduced.

CONDENSED PROTOCOL

Dog 241. Male. Weight 6.92 kgm.

October 21, 1918. Right adrenal excised. It weighed 0.55 gram and contained 0.47 mgm. epinephrin. Nerves of left adrenal cut (major and minor splanchnics, and all twigs seen going to the adrenal).

November 11, 1918. Condition excellent, weight 7.5 kgm.

Under morphine and ether, obtained a specimen of blood from the jugular vein. Then made cava pocket in usual way, tying abdominal aorta but not the coeliac and mesenteric arteries.

Collected the following specimens of adrenal blood:
1st specimen, 2.1 grams in 1½ minutes (1.4 grams per minute)
2nd specimen, 6.85 grams in 5 minutes (1.37 grams per minute)
3rd specimen, 6.0 grams in 5 minutes (1.2 grams per minute)
4th specimen, 3.3 grams in 4 minutes (0.8 gram per minute)
Now obtained additional indifferent blood from the jugular and a sample from the abdominal aorta. The left adrenal weighed 0.562 gram and contained 0.66 mgm. epinephrin.

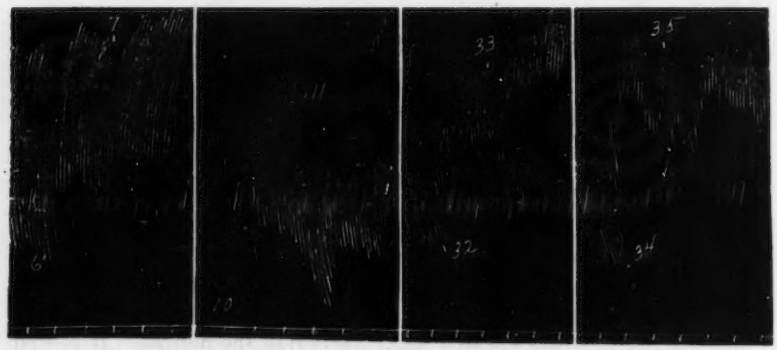

Fig. 1. Intestine tracings. Bloods from dog 241. At *6*, Ringer was replaced by jugular blood and this at *7* by the 4th adrenal specimen; at *10* Ringer was replaced by jugular blood, and this at *11* by jugular blood to which was added adrenalin to make a concentration of 1: 62,500,000. The bloods were diluted with three volumes Ringer (the adrenalin blood after adding the adrenalin); at *32* Ringer was replaced by jugular blood and this at *33* by the 3rd adrenal specimen; at *34* Ringer was replaced by jugular blood and this at *35* by jugular blood to which was added adrenalin to make a concentration of 1 : 95,000,000. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). As in all the other figures, time is marked in half-minutes. (Reduced to one-half.)

The epinephrin assay of the adrenal blood (samples of the tracings are given in fig. 1) showed that the 4th specimen was much weaker than 1: 62,500,000 adrenalin[1] (observations 7 and 11). Other tracings,

[1] All our results on the concentration and output of epinephrin are given in terms of the base, the amount of the base in Parke, Davis & Co.'s adrenalin hydrochloride solution being assayed by the colorimetric method of Folin, Cannon and Denis. The assay of freshly opened bottles has usually yielded from 65 to 80 per cent adrenalin base. A bottle which is used more than once is always re-assayed from time to time.

not reproduced, indicated that it did not differ much from 1: 125,000,000. The 2nd and 3rd specimens, corresponding to the greater flow, were weaker than the 4th, the 2nd being decidedly weaker than 1: 125,000,000 adrenalin. This was confirmed by several observations (not reproduced). In figure 1 it is shown that the 3rd specimen was distinctly weaker than 1: 95,000,000 adrenalin (observations 33 and 35).

TABLE 1

NUMBER OF DOG	BODY WEIGHT	WEIGHT OF ADRENALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
			Grams	Minutes	Seconds	Grams per minute		Per animal per minute	Per kilogram per minute
	kgm.	*grams*						*mgm.*	*mgm.*
15	8.5		24	3		8.0	1: 3,500,000	0.0024	0.0002
			22	3		7.3	1: 3,000,000	0.0024	0.0002
17	6.7	0.65	20	4	45	4.2	1: 3,300,000	0.0013	0.0002
18	7.7	0.78	13	2		6.5	1: 6,000,000	0.001	0.00015
206	6.2	0.905	22.9	2		11.5	1: 7,500,000	0.0015	0.00025
207	7.3	1.2	16.1	2		8.0	1: 8,000,000	0.001	0.00014
221	11.3	1.8	17.4		30	34.8	1: 18,000,000	0.002	0.00018
245	9.5	1.55	10.0	1		10.0	1: 3,750,000	0.0027	0.00028
246	7.5	1.26	7.7		30	15.4	1: 13,000,000	0.0012	0.00016
247	7.25	1.12	4.8	1	30	3.2	1: 1,800,000	0.0018	0.00025
248	4.6	0.8	9.7	1		9.7	1: 13,000,000	0.0008	0.00017
249	6.6	0.848	9.6	1		9.6	1: 6,250,000	0.0015	0.00023
256	6.2	0.733	10.6	1	30	7.1	1: 6,500,000	0.0011	0.00018
257	6.0	0.908	8.65	1		8.65	1: 6,650,000	0.0013	0.0002
263	4.4	0.78	6.15	1		6.15	1: 3,800,000	0.0016	0.00036
280	7.0	1.8	12.5	1		12.5	1: 10,000,000	0.0013	0.00018
306	5.05	0.705	8.2	1		8.2	1: 8,000,000	0.001	0.0002
307	4.6	0.831	11.05	1	30	7.4	1: 4,800,000	0.0015	0.00033

The first three dogs in the table were anesthetized with ether alone, the rest with morphine and ether.

Taking the 4th adrenal specimen at 1: 120,000,000 we get an output of 0.0000065 mgm. per minute for the dog, or 0.0000009 mgm. per kilogram of body weight per minute. The normal average output for dogs under the conditions of our experiments, as assayed on rabbit intestine (and uterus) segments, is 0.00022 mgm. per kilogram per minute (table 1). The output had, therefore been reduced by the operation in this animal to less than $\frac{1}{200}$ of the normal.

CONDENSED PROTOCOL

Dog 242. Female. Weight 5.21 kgm.

October 28, 1918. Right adrenal excised. It weighed 0.425 gram and contained 0.47 mgm. epinephrin. Nerves of left adrenal cut (major and minor splanchnics and all branches seen entering gland; superior mesenteric ganglion and part of coeliac ganglion also excised).

November 22, 1918. Condition excellent. Weight 5.6 kgm. Under morphine and ether collected jugular blood, then made cava pocket in usual way, tying abdominal aorta but not the coeliac and mesenteric arteries.

Collected adrenal blood:

11.35 a.m. 1st specimen 0.6 gram in 30 seconds (1.2 grams per minute). 2nd specimen 6.15 grams in 5 minutes (1.2 grams per minute)

11.55 a.m. Injected intravenously 0.3 mgm. strychnine sulphate—clonic spasms

11.56 a.m. 3rd specimen, 0.75 gram in 30 seconds (1.5 grams per minute). 4th specimen, 6.05 grams in 4 minutes (1.5 grams per minute)

12.36 p.m. 5th specimen, 0.55 gram in 30 seconds (1.1 grams per minute). 6th specimen, 5.9 grams in 6 minutes (1.0 gram per minute)

Now obtained more indifferent blood from abdominal aorta.

Left adrenal weighed 0.491 gram and contained 0.58 mgm. epinephrin

The epinephrin assay of the adrenal vein blood (a few of the tracings are reproduced in fig. 2) gave no indication that any epinephrin whatever was present, although the segments used for the assay were more than usually sensitive. We have shown elsewhere (3) that strychnine causes a marked and sustained increase in the rate of liberation of epinephrin, doubtless through the nervous mechanism. Yet neither before nor after strychnine could any epinephrin be detected in the adrenal vein blood. It was found that with indifferent blood made up to a concentration of 1: 165,000,000 adrenalin and then diluted with three volumes of Ringer's solution, adrenalin was easily detected. Indifferent blood made up to a concentration of 1: 330,000,000 adrenalin and then diluted with its own volume of Ringer produced a definite inhibition of the intestine segment. When the weight on the lever was increased the reaction became still more delicate, so that indifferent blood made up with adrenalin to a concentration of 1: 500,000,000 and then diluted with an equal volume of Ringer's solution gave a distinct effect (observation 29, fig. 2), as did a concentration of 1: 650,000,000 (observation 33). Even a concentration of 1: 800,000,000 could be plainly detected (observation 35, fig. 2), while the 6th adrenal blood specimen similarly diluted gave no inhibition whatever (observation 31). Numerous additional observations (not reproduced) were made, but no evidence was obtained that any of the adrenal specimens contained epinephrin.

The output could not have been as much as 0.000001 mgm. per minute for the dog, or 0.0000002 mgm. per kilogram per minute, i.e. not $\frac{1}{1000}$ of the normal output. The section of the nerves was, therefore, complete. The failure of strychnine to excite any detectable secretion confirms this conclusion.

Although in these experiments the condition of the animals at the time they were sacrificed for the determination of the residual output of epinephrin was so good that there could be no reasonable doubt that they would have survived indefinitely, the next dog was allowed

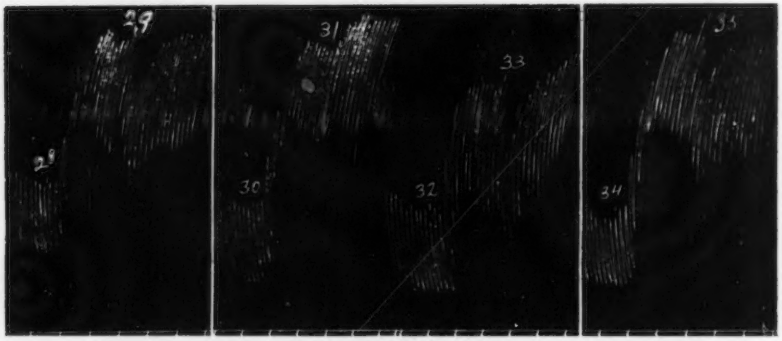

Fig. 2. Intestine tracings. Bloods from dog 242. At *28*, *30*, *32* and *34* Ringer was replaced by indifferent blood to which was added adrenalin to make a concentration of 1: 500,000,000; at *31* by the 6th adrenal specimen; at *33* by indifferent blood to which was added adrenalin to make a concentration of 1: 650,000,000 and at *35* by indifferent blood to which was added adrenalin to make a concentration of 1: 800,000,000. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

to live for a longer period (eighty days). For the determination of the degree in which the epinephrin output has been suppressed by the operation this longer survival period has one disadvantage, namely, the possibility that regeneration of the secretory fibers may have occurred before the residual output of epinephrin is estimated.

CONDENSED PROTOCOL

Dog 243. Male. Weight 6.5 kgm.

October 28, 1918. Right adrenal excised. It weighed 0.428 gram. Nerves of left adrenal cut as in dog 241. The animal made a rapid recovery.

January 16, 1919. Condition excellent. Weight 7.6 kgm.

Under morphine and ether obtained a sample of jugular blood. Then made cava pocket as in the other dogs, and collected adrenal blood.

12.45 p.m. 1st specimen, 2.05 grams in 30 seconds (4.1 grams per minute). 2nd specimen, 8.35 grams in 2 minutes (4.1 grams per minute)

12.50 p.m. Injected 0.5 mgm. strychnine sulphate intravenously. Tonic convulsions. Artificial respiration started. Collected adrenal blood.

12.50½ p.m. 3rd specimen, 1.15 grams in 30 seconds (2.3 grams per minute). 4th specimen, 5.5 grams in 2 minutes (2.75 grams per minute)

1.05 p.m. Convulsions ceased; spontaneous respiration going on, with hyperpnoea. Stopped artificial respiration.

1.15 p.m. Reflexes still exaggerated. Collected adrenal blood. 5th specimen, 2.5 grams in 45 seconds (3.3 grams per minute). 6th specimen, 6.9 grams in 2 minutes (3.45 grams per minute)

Now obtained blood from abdominal aorta. Left adrenal weighed 0.49 gram.

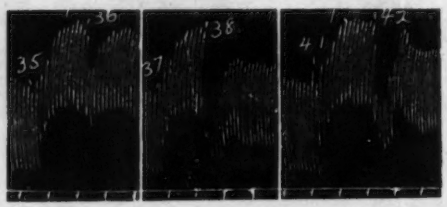

Fig. 3. Intestine tracings. Bloods from dog 243. At *35*, *37* and *41* Ringer was replaced by jugular blood and this at *36* by jugular blood to which was added adrenalin to make a concentration of 1: 10,000,000; at *38* by jugular blood to which was added adrenalin to make a concentration of 1: 8,000,000; at *42* by the 2nd adrenal specimen. All of the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

The epinephrin assay showed that the 2nd adrenal specimen had a concentration less than 1: 7,000,000 and greater than 1: 10,000,000 (fig. 3, observations 36 and 42—confirmed by several pairs of observations not reproduced). Observation 38 (fig. 3) indicates that the 2nd specimen was somewhat less than 1: 8,000,000. The 4th specimen (taken after strychnine injection) had a much greater concentration than 1: 7,000,000 and was stronger than 1: 4,000,000 (fig. 4, observations 44 to 56). Figure 5 (observations 58, 60 and 62) shows that the 4th specimen did not differ much from 1: 2,800,000. It was decidedly stronger than 1: 4,000,000 and much weaker than 1: 1,300,000 (fig. 5, observation 64). Qualitatively the 4th specimen was found to be much stronger than the 2nd (intestine observations not reproduced, confirmed by uterus observations 80 and 81, fig. 6). The assay indicates that the difference between the 4th and 2nd specimens is more

than can be accounted for by the difference in the blood-flows. Taking the 2nd specimen at 1: 9,000,000, we get 0.00045 mgm. per minute for the dog, or 0.00006 mgm. per kilogram per minute, i.e., $\frac{1}{3}$ to $\frac{1}{4}$ of the

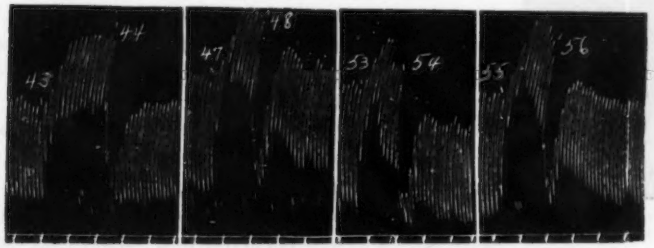

Fig. 4. Intestine tracings. Bloods from dog 243. At *43*, *47*, *53* and *55* Ringer was replaced by jugular blood, and this at *44* and *54* by the 4th adrenal specimen; at *48* by jugular blood to which was added adrenalin to make a concentration of 1: 7,000,000; at *56* by jugular blood to which was added adrenalin to make a concentration of 1: 4,000,000. All the bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

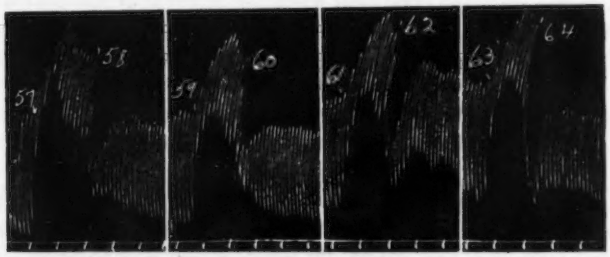

Fig. 5. Intestine tracings. Bloods from dog 243. At *57*, *59*, *61* and *63* Ringer was replaced by arterial blood and this at *58* by arterial blood to which was added adrenalin to make a concentration of 1: 2,800,000; at *60* by the 4th adrenal specimen; at *62* by arterial blood to which was added adrenalin to make a concentration of 1: 4,000,000; at *64* by arterial blood to which was added adrenalin to make a concentration of 1: 1,300,000. The bloods were diluted with one volume Ringer (the adrenalin bloods after adding the adrenalin). (Reduced to one-half.)

normal output for both adrenals. This would correspond to $\frac{1}{2}$ to $\frac{2}{3}$ the normal output for the denervated adrenal. As we have never found so great an output after this operation when the assay was made two or three weeks after the operation, the suggestion is that some

regeneration must have occurred. This conclusion is strengthened by the fact that strychnine seems to have increased the output. Thus, if the 4th specimen is taken at 1:2,800,000, the output per minute after strychnine would be 0.001 mgm. for the dog or 0.00013 mgm. per kilogram per minute, about double the initial output. The increase is not very great compared with that usually caused by strychnine in a normal animal, but this may be because regeneration was incomplete at the time the animal was sacrificed. In any case we should not venture to draw from this experiment the definite conclusion that regeneration of these fibers had occurred to a substantial extent in the eighty days since the operation. It is always possible in the present state of our knowledge of these secretory paths that owing to some anomaly a large portion of the fibers was spared by our operation. Our only ground for considering this to be improbable is that we have not encountered the condition when the interval of survival was only a few weeks.

Fig. 6. Uterus tracings. Bloods from dog 243. At *80* Ringer was replaced by the 4th adrenal specimen; at *81* by the 2nd adrenal specimen. The bloods were diluted with ten volumes Ringer. (Reduced to one-half.)

The normal output of epinephrin was estimated in two monkeys.

CONDENSED PROTOCOL

Macacus cynomolgos.[2] *Female. Weight 2.51 kgm.*

Under morphine and urethane a sample of blood was drawn from the internal jugular vein. Then the cava pocket was made as in the other experiments, the abdominal aorta being tied, but not the coeliac or mesenteric arteries. Adrenal blood was collected:

1st specimen, 3.4 grams in 1 minute (3.4 grams per minute)

2nd specimen, 7.0 grams in 3 minutes (2.33 grams per minute)

3rd specimen, 4.95 grams in 3 minutes (1.65 grams per minute)

More jugular and some arterial blood was now obtained. The right adrenal weighed 0.286 gram, the left 0.283 gram.

It was found at autopsy that a vein coming from the upper pole of the right adrenal passed up through the liver before entering the cava. The blood from this vein which, from its size, probably carried about one-third of the blood from

[2] In a previous paper this animal, an immature specimen showing complete permanent set of teeth with exception of the third molars above and below, was incorrectly referred to as a baboon.

that adrenal, would not be collected in clipping off the cava pocket. This vein was seen in the other monkeys operated on and must always be taken account of. The main adrenal vein from the right gland entered the cava at about the level usual in the dog and the blood from it could be collected.

It was shown on rabbit segments that the 3rd specimen was stronger than the 2nd, corresponding to the smaller flow; that the 2nd and 3rd specimens were stronger than 1:7,000,000 and weaker than 1:2,750,000 adrenalin; that the 3rd specimen was somewhat stronger than 1:4,800,000 and somewhat weaker than 1:4,000,000 while the 2nd specimen was somewhat weaker than 1:5,500,000.

Taking the 2nd specimen at 1:6,000,000 we get an output of 0.0004 mgm. per minute for the animal, or correcting for the loss of a portion of the blood from the right adrenal, say 0.0005 mgm., i.e., 0.0002 mgm. per kilogram of body weight per minute. Taking the 3rd specimen at 1:4,300,000, we get the same output.

In another monkey (Macacus rhesus) weighing 8.4 kilograms the output with an adrenal blood flow of 7.5 grams per minute was 0.0013 per minute for the animal, or 0.00015 mgm. per kilogram per minute. The right adrenal weighed 0.465 gram. The left adrenal weighed 0.48 gram.

In two monkeys (Macacus rhesus) the adrenal operation (excision of the right gland, denervation of the left) was performed in the usual manner. One of the animals was sacrificed after twenty-two days for estimation of any residual epinephrin output. It was at that time in excellent condition and, so far as could be made out, unaffected in any way by the loss of the epinephrin secretion. The other monkey was allowed to survive and is still alive and well five months after the operation. His weight is precisely the same as at the time of the operation. The condensed protocol of the monkey which was sacrificed follows:

CONDENSED PROTOCOL

Macacus rhesus. Male.

October 29, 1918. Right adrenal excised. It weighed 0.325 gram and contained 0.52 mgm. epinephrin. The left adrenal was denervated. Recovery was rapid, and the animal was active the next day.

November 20, 1918. Condition excellent. Weight 4.1 kgm.

Under urethane obtained indifferent (internal jugular) blood. Then made cava pocket as in the other animals. Collected adrenal blood.

1st specimen, 2.8 grams in 1 minute (2.8 grams per minute)

2nd specimen, 5.45 grams in 2 minutes (2.72 grams per minute)

3rd specimen, 7.05 grams in 3 minutes (2.35 grams per minute)

4th specimen, 5.75 grams in 3 minutes (1.91 grams per minute)
Now obtained more venous blood through cannula pushed into right heart through inferior cava and also some arterial blood. Left adrenal weighed 0.442 gram and contained 0.66 mgm. epinephrin.

With the rabbit intestine and uterus segments none of the adrenal specimens gave any reaction from which the presence of epinephrin could be deduced. Samples of the tracings are reproduced in figures 7 to 9. In figure 7 it is shown that the 4th specimen could not have contained epinephrin even in a concentration of 1: 130,000,000. Even with the undiluted blood the 3rd specimen produced no definite inhibition of the intestine (fig. 8, observation 22). Another test with the 3rd specimen immediately after observation 30 gave an absolutely negative result. Yet the segment gave a distinct reaction with 1: 330,000,000 adrenalin in indifferent blood (observation 30), a stronger reaction with 1: 160,000,000 and a marked effect with 1: 80,000,000.

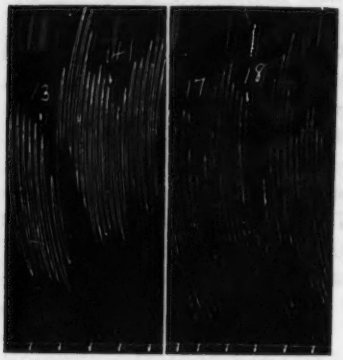

Fig. 7. Intestine tracings. Bloods from monkey, whose right adrenal was excised and left adrenal denervated 22 days prior to experiment. At *13* and *17* Ringer was replaced by venous blood and this at *14* by the 4th adrenal specimen; at *18* by venous blood to which was added adrenalin to make a concentration of 1: 130,000,000. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

It was confirmed on the uterus that the adrenal specimens caused effects which did not differ sufficiently from those produced by indifferent blood to permit the conclusion that they contained epinephrin. Thus, the increase of tone produced by the 4th specimen was little greater than that produced by indifferent (arterial) blood (fig. 9, observations 33 and 34). The effect caused by the 3rd specimen was no greater than that caused by indifferent (venous) blood, although adrenalin in a concentration of 1: 80,000,000 in the indifferent blood gave a good rise of tone (fig. 9, observations 43 to 45).

Since our last paper (1) on this subject was published we have had the opportunity, in connection with other researches, of estimating the residual output of epinephrin after excision of one adrenal and denerva-

tion of the other in a considerable number of cats, which were allowed to survive long enough to recover completely from the effects of the operation. The results are combined in table 2, which supplements the similar data already published in tabular form in a previous paper (4).

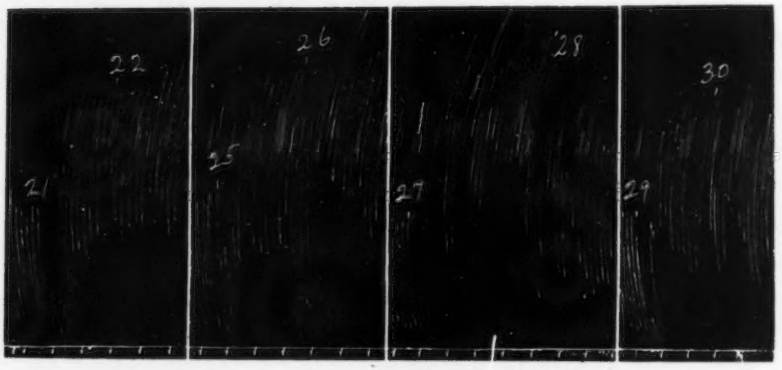

Fig. 8. Intestine tracings. Bloods from same monkey as in figure 7. At *21*, *25*, *27* and *29* Ringer was replaced by arterial blood and this at *22* by the 3rd adrenal specimen; at *26* by arterial blood to which was added adrenalin to make a concentration of 1: 160,000,000; at *28* by arterial blood with adrenalin to make a concentration of 1: 80,000,000; at *30* by arterial blood with adrenalin added to make a concentration of 1: 330,000,000. The bloods were not diluted before application to the segment. (Reduced to one-half.)

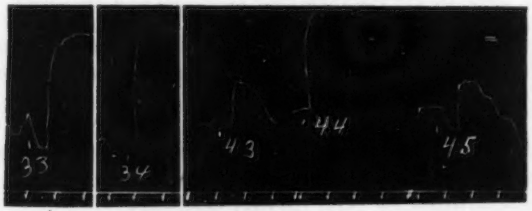

Fig. 9. Uterus tracings. Bloods from same monkey as in figures 7 and 8. At *33* Ringer was replaced by arterial blood; at *34* by the 4th adrenal specimen. The bloods were diluted with five volumes Ringer. With another uterus segment, at *43* Ringer was replaced by the 3rd adrenal specimen; at *44* by venous blood with adrenalin added to make a concentration of 1: 80,000,000, and at *45* by venous blood. The bloods were diluted with one volume Ringer (the adrenalin blood after adding the adrenalin). (Reduced to one-half.)

Five cats, not included in the table, were sacrificed in experiments which did not permit an assay of the adrenal blood. They had survived for a period of from ten to sixty-two days in good health.

Since it is important for estimating the degree in which the epinephrin output has been affected by denervation or by any other procedure, to establish on the basis of as large a number of observations as possible

TABLE 2

NUMBER OF CAT	BODY WEIGHT	EPINEPHRIN OUTPUT		FRACTION OF NORMAL LIBERATION	DAYS AFTER OPERATION
		Per minute	Per kilogram per minute		
	kgm.	*mgm.*	*mgm.*		
201	2.575	0.000012	0.0000045	$\frac{1}{50}$	18
202	2.545	0.000007	0.000003	$\frac{1}{100}$*	8
215	2.38	0.00007	0.00003	$\frac{1}{10}$	59
219	2.54	0.000046	0.000018	$\frac{1}{12}$	27
231	3.08	0.00003	0.00001	$\frac{1}{25}$*	33
233	2.62	0.000033	0.000013	$\frac{1}{20}$	36
235	2.25	0.000002	0.0000009	$\frac{1}{250}$*	40
277	1.75	0.000055	0.00003	$\frac{1}{8}$†	21
304	2.15	0.000033	0.000016	$\frac{1}{15}$	46

* In these cats there was no evidence that any epinephrin was being liberated. The amount given in the table is such as could have been detected by the test objects.

† Strychnine was administered before collection of the adrenal blood; this may have increased the output, so that the amount indicated by the assay is probably higher than the actual residual liberation.

In cat 215, assay by eye reactions gave an output of 0.00014 mgm. per minute for the animal, or 0.00006 mgm. per kgm. per minute, i.e., about $\frac{1}{3}$ of the normal average. In cat 233, assay by eye reactions gave an output of 0.00006 mgm. per minute for the animal, or 0.000023 mgm. per kilogram per minute, i.e., $\frac{1}{25}$ of the normal average.

the normal average output and the normal range, additional data on cats are collected in table 3. All the assays given in tables 1 to 3 were made on rabbit intestine (and uterus) segments.

The average output of epinephrin per kgm. of body weight per minute for the urethanized cats is 0.00025 mgm., the same as that given in the previous paper (1). The average for the 16 etherized cats is 0.0002 mgm. The average for the 45 cats included in table 2 of the previous paper and table 3 of this paper is 0.00023 mgm. per kgm. per minute.

TABLE 3

NUMBER OF CAT	BODY WEIGHT	WEIGHT OF ADRENALS	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
			Grams	Minutes	Seconds	Grams per minute		Per animal per minute	Per kilogram per minute
	kgm.	*grams*						*mgm.*	*mgm.*
204	3.25	0.60	6.4		30	12.8	1: 14,000,000	0.0009	0.00027
205	2.7	0.68	7.3	3		2.4	1: 4,000,000	0.0006	0.0002
210	2.9	0.583	3.7	7		0.5	1: 800,000	0.0006	0.0002
211	2.8	0.58	3.5	4		0.9	1: 2,000,000	0.0005	0.0002
212	2.2	0.496	4.3	3		1.43	1: 3,200,000	0.00045	0.0002
213	2.67	0.468	9.8	3		3.3	1: 3,500,000	0.001	0.00037
214	2.35	0.348	7.0	3		2.3	1: 2,000,000	0.0011	0.00045
222	2.17	0.298	7.0	3		2.3	1: 5,500,000	0.0004	0.0002
223	3.47	0.462	6.1	2	30	2.45	1: 2,500,000	0.001	0.0003
225	2.05	0.292	3.7	3		1.2	1: 2,400,000	0.0005	0.00025
228	2.5	0.492	5.2	1	30	3.5	1: 5,800,000	0.0006	0.00024
229	2.07	0.387	4.7	3		1.6	1: 2,200,000	0.0007	0.00035
230	2.08	0.412	4.75	3		1.6	1: 2,500,000	0.0006	0.0003
238	1.64	0.288	5.3	3		1.7	1: 5,000,000	0.00034	0.0002
239	2.41	0.35	4.6	2		2.3	1: 5,000,000	0.00046	0.0002
258	2.76	0.323	6.05	3		2.0	1: 4,800,000	0.00042	0.00015
259	4.1	0.73	9.05	2		4.5	1: 4,500,000	0.001	0.00025
281	4.37	0.553	7.8	2		3.9	1: 4,500,000	0.00087	0.0002
282	3.1	0.51	5.7	2		2.9	1: 3,000,000	0.0009	0.0003
283	3.38	0.33	5.55	2		2.8	1: 7,000,000	0.0004	0.00012
284	2.85	0.502	4.0	2		2.0	1: 7,000,000	0.0003	0.0001
285	4.4	0.553	9.2	2		4.6	1: 6,500,000	0.0007	0.00016
286	4.0	0.372	7.1	1	30	4.7	1: 6,500,000	0.00072	0.00018
287	3.71	0.376	4.8	3		1.6	1: 6,500,000	0.00035	0.0001
288	2.175	0.37	4.2	2		2.1	1: 3,800,000	0.00055	0.00025
289	2.83	0.342	6.0	3		2.0	1: 1,300,000	0.0015	0.0005
290	2.265	0.296	3.3	3		1.1	1: 2,000,000	0.00055	0.00025
292	2.07	0.278	2.35	5		0.5	1: 1,300,000	0.0004	0.0002
293	3.865	0.377	8.8	1	30	5.87	1: 9,000,000	0.00065	0.00017
294	2.73	0.316	6.85	1	30	4.8	1: 6,600,000	0.0007	0.00025
295	2.9	0.372	6.45	2		3.2	1: 8,800,000	0.0005	0.00017
296	2.0	0.215	3.7	4		0.92	1: 2,300,000	0.0004	0.0002
298	3.35	0.418	5.35	3		1.8	1: 3,000,000	0.0006	0.00018
303	4.82	0.66	9.05	1	30	6.0	1: 6,000,000	0.001	0.0002
305	3.41	0.312	6.8	2		3.4	1: 6,000,000	0.00057	0.00017

The first 19 cats in the table (down to and including cat 282) were anesthetized with urethane, the rest with ether.

CONCLUSION

In dogs and monkeys, as previously shown for cats and rabbits, the liberation of epinephrin from the adrenals is not indispensable for life and health.

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THE GASTRIC RESPONSE TO FOODS

II. A Fractional Study of the Coagulation of Milk in the Human Stomach[1]

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In the course of our experiments upon the gastric response to foods, we were particularly fortunate in securing the coöperation of a normal man, thirty-one years of age, who had the unique ability to deliver samples of stomach contents at will. Not only could this man completely empty his stomach at any desired stage in the process of digestion but he could also give us small samples at short intervals throughout the entire gastric cycle. In other words, we could make a fractional examination of his stomach contents without recourse to a stomach tube. Inasmuch as this regurgitator was to be available for only a short time, we decided to study milk coagulation at the outset of our observations. These studies told us much of interest. For example, we learned that raw whole cows' milk forms a large hard curd whereas boiled milk curds in a much finer and softer form; that the presence of much cream (milk fat) in the milk insures the formation of particularly soft curds which are slow to leave the stomach; that skimmed milk yields a particularly hard curd due to the absence of fat; that pasteurized milk shows smaller curds than the raw whole milk but larger than the boiled whole milk; that cold milk coagulated more slowly than warm milk, and that milk drunk very slowly, or "sipped," curds in larger curds and leaves the stomach more slowly than similar milk drunk very rapidly.

The only other experiments in which a regurgitator was used in the study of milk curding, so far as we are aware, are those of Brennemann (1). However in the case of Brennemann's work no attempt was made to follow the milk through the various stages of its coagula-

[1] The expenses of this investigation were defrayed by a fund furnished by Mrs. M. H. Henderson, the Curtis Publishing Co. and Dr. L. M. Halsey.

tion, the experimental procedure simply embracing the *complete* emptying of the stomach at a certain time (usually thirty minutes) after drinking the milk. He did, however, get a line upon the curding sequence in certain cases by emptying the stomach at different intervals upon successive days. In spite of the variation in our plan of experimentation we have nevertheless been able, wherever our investigation overlapped that of Brennemann, to verify him in remarkable detail.

EXPERIMENTAL

Studies of two kinds were made—one in which small samples of stomach contents were regurgitated at intervals throughout the curding period of the milk, the other in which the stomach was completely emptied a half-hour after a pint of milk had been ingested. Unless otherwise stated the experiments were made before breakfast on an empty stomach. Typical protocols follow:

Experiment 1. Raw whole milk.

Five hundred cubic centimeters of milk with a temperature of 37°C. were drunk in thirty seconds. Eleven samples were regurgitated at intervals for one hour, the first regurgitation taking place one-half minute after the milk was taken.

Remarks. Small curds were regurgitated one-half minute after the milk was drunk. These curds were white in color and those returned thirty to forty minutes after drinking the milk were firm and rubber-like in texture and as large as an average size peanut. At the end of the hour tough curds as large as a man's thumb were in evidence. (See exper. 14. For photographs of curds, see fig. 1a).

Experiment 2. Boiled whole milk.

Five hundred cubic centimeters of milk were boiled for five minutes, then cooled to 37°C. and drunk in ten seconds. Ten samples were regurgitated at intervals for one hour, the first one at the one-minute period.

Remarks. In the case of boiled milk the curds were much smaller and softer than were the curds from raw whole milk. They were also not white as were the raw milk curds but possessed a yellowish tint. The maximum size of the boiled milk curds was about that of a small pea. (See fig. 2).

Experiment 3. Raw skimmed milk.

Five hundred cubic centimeters of milk, temperature 37°C., were taken in ten seconds. Ten samples were regurgitated, the first being returned thirty seconds after the milk was drunk.

Remarks. Large curds formed more quickly from skimmed milk than from whole milk. In one-half minute after the milk was drunk, small curds began to form. Within five minutes white curds as large as a man's thumb were regurgitated. These curds were firmer and more rubber-like than those from any other kind of milk tested. (For pictures see fig. 3).

Experiment 4. Influence of water.

In this test 500 cc. distilled water (temp. 25°C.) were drunk in five minutes, and after an interval of three minutes 500 cc. of raw whole milk (temp. 37°C.) were also drunk in five minutes. Eleven samples were regurgitated during the

next hour, the first one being returned thirty seconds after the drinking of the milk.

Remarks. The diluted milk coagulated about the same as the undiluted milk except that the maximum curd formation was hardly as large (See exper. 1). (For photographs of curds, see fig. 4).

Experiment 5. Influence of sodium bicarbonate.

Five hundred cubic centimeters of raw whole milk (temp. 37°C.) to which had been added 2.5 grams of sodium bicarbonate were drunk in thirty seconds. Eleven samples were regurgitated, the first one at the half-minute interval.

Remarks. In spite of the fact that the stomach contents were alkaline up to one-half hour after the milk was drunk, definite curds began to form at the five-minute interval. The curds were smaller and softer than the curds of raw whole milk (exper. 1) but not so soft and flaky as were the curds from the boiled whole milk (exper. 2). (For pictures of curds see fig. 5).

Experiment 6. Cream (20 per cent fat).

Five hundred cubic centimeters of cream containing 20 per cent milk fat (temp. 37.5°C.) were drunk in ten seconds. Nine samples were regurgitated at intervals for two hours and a half, the first sample being collected one-half minute after drinking the cream.

Remarks. Small soft curds reaching their maximum size at about twenty minutes (see fig. 6). Stomach emptied slowly due to presence of fat. (See exper. 11).

Experiment 7. Mixture of boiled and raw milk.

Four hundred cubic centimeters of raw whole milk were boiled for five minutes, then cooled to 37°C. To this was added 100 cc. of the original raw milk and the mixture drunk in ten seconds. Eleven samples were regurgitated in an hour, the first one at the one-half minute interval.

Remarks. Notwithstanding the fact that 80 per cent of the milk was *boiled*, the mixture curded more after the manner of *raw* milk. Fairly large curds were formed in twenty minutes. At no time were the soft, fluffy curds of boiled milk in evidence. (See fig. 7).

Experiment 8. Influence of water.

In this test 500 cc. distilled water (temp. 37°C.) were drunk in fifteen seconds, followed immediately by 500 cc. raw whole milk of same temperature, also drunk in fifteen seconds. Twelve samples were collected during the following hour, the first one being regurgitated at the one-half minute interval.

Remarks. There was less curd formation in this experiment than in experiment 4 and the stomach apparently emptied somewhat more slowly.

Experiment 9. Boiled skimmed milk.

Five hundred cubic centimeters of skimmed milk (sp. gr. 1034 at 10°C.) were boiled for five minutes, then cooled to 37°C. and drunk in ten seconds. Eleven samples were subsequently regurgitated within one hour, the first sample being returned in one-half minute from the time the milk was ingested.

Remarks. Curds began to form in one-half minute, but these curds were smaller than in the case of raw skimmed milk (see exper. 3). In fact they never became larger than a small kidney bean. The curds were, however, larger than those regurgitated from the boiled whole milk (see exper. 2). (For photographs see fig. 8).

Experiment 10. Pasteurized milk.

Five hundred cubic centimeters of pasteurized whole milk (sp. gr. 1029 and temp. 20°C.) were drunk in ten seconds. Eleven samples were collected in one hour, the first specimen being regurgitated one-half minute after the drinking of the milk.

Remarks. Small curds, formed at the two and one-half minute period. Maximum curd formation at forty minutes. The curds were intermediate in character between those of raw and boiled milk, but more nearly approached the raw milk type. (See curds in fig. 9).

Experiment 11. Cream (40 per cent fat).

Five hundred cubic centimeters of pasteurized cream (40 per cent fat) having a temperature of 37.5°C. were drunk in ten seconds. Fourteen samples were collected during an interval of two and one-half hours, the first sample being regurgitated at the one-half minute interval.

Remarks. No curd formation until one-half hour after the cream was drunk. Even then the curds were very small (pinhead) and did not increase in size thereafter. The stomach emptied very slowly and two and one-half hours after 500 cc. of the cream were drunk, 475 cc. of material were regurgitated (see exper. 6). (For photographs see fig. 10).

Experiment 12. Milk drunk slowly.

Five hundred cubic centimeters of raw whole milk at 20°C. were drunk in *ten minutes* and the stomach completely emptied by regurgitation one-half hour later. (For photograph of the curds see figs. 11 and 12). Experiment 13 followed immediately.

Remarks. There was more curd regurgitated one-half hour after 500 cc. of milk were drunk slowly than when a similar volume of milk was taken rapidly. The individual curds were also larger. (See expers. 13, 15 and 16).

Experiment 13. Milk drunk rapidly.

Five hundred cubic centimeters of raw whole milk at 20°C. were drunk in *fifteen seconds* and the stomach completely emptied by regurgitation one-half hour later. (For photograph of the curds see figs. 11 and 12).

Remarks. The total mass of curd regurgitated one-half hour after drinking 500 cc. of milk rapidly was less than after a similar volume of milk had been drunk slowly. The curds were also smaller. (See expers. 12, 15 and 16).

Experiment 14. Raw whole milk.

Five hundred cubic centimeters of raw whole milk, 20°C., drunk in seventeen seconds. Eight samples were regurgitated in forty minutes, the first one being collected one minute after drinking the milk.

Remarks. Curds began to form in one minute and in twenty-five minutes a curd weighing 15 grams was regurgitated. This curd was very tough and "rubbery." (See exper. 1). (For pictures of curds see fig. 1).

Experiment 15. Milk drunk rapidly.

Five hundred cubic centimeters raw whole milk, 20°C., drunk in fifteen seconds. One-half hour later the stomach was completely emptied by regurgitation. Experiment 16 followed immediately.

Remarks. This experiment is a duplicate of experiment 13 except that it preceded the test on the slow drinking of milk (see exper. 16) instead of following that test as in the case of experiment 13. At the end of the experiment 160 cc.

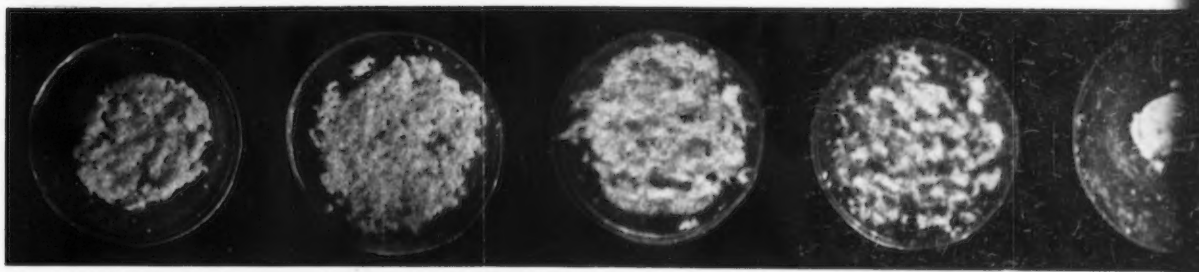


Fig. 1. Five hundred cubic centimeters whole raw cows' milk, drunk in 17 seconds. Regurgitation after 1, 2, 3, 5, 10 an
Milk 20°C. (0.35 natural size.)

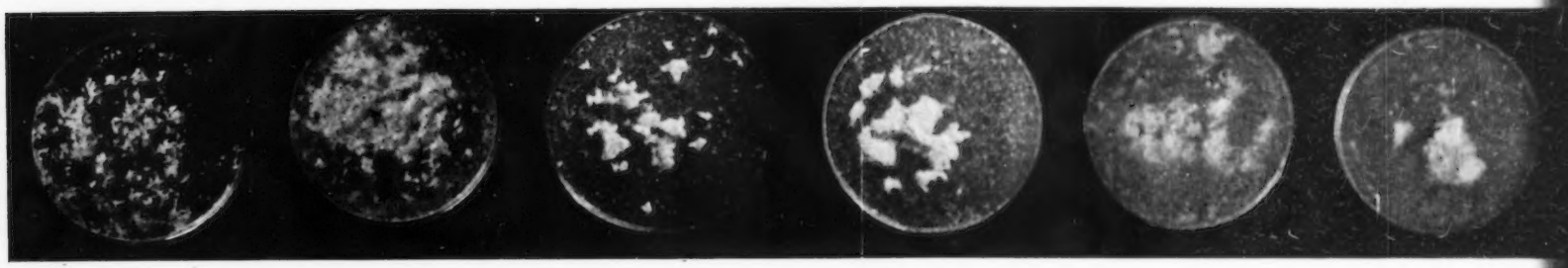


Fig. 1a. Five hundred cubic centimeters raw whole milk at 37°C., drunk in 30 seconds. Regurgitation after 0.5, 1, 2.5, 5, 10, 15, 30 and 40 m

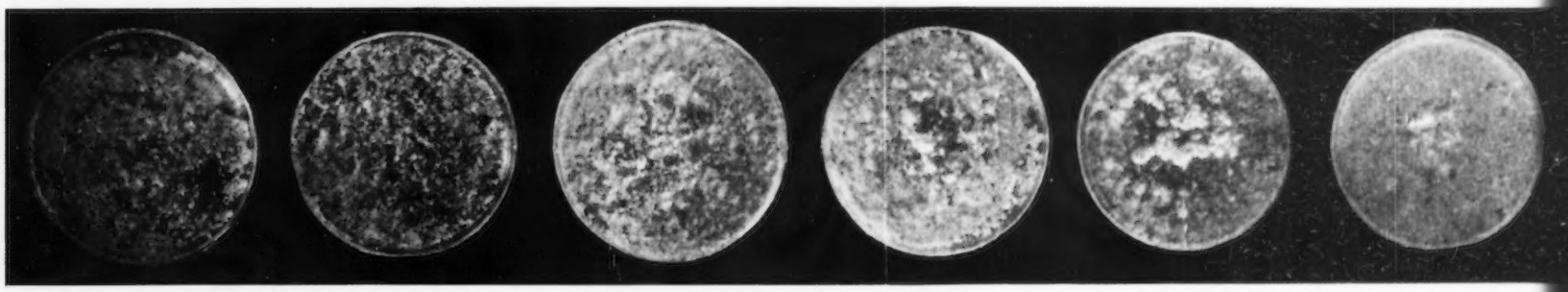


Fig. 2. Five hundred cubic centimeters whole cows' milk boiled 5 minutes. Drunk in 10 seconds. Regurgitation after 1, 3, 7, 11, 15, 39, 55 and 60 min

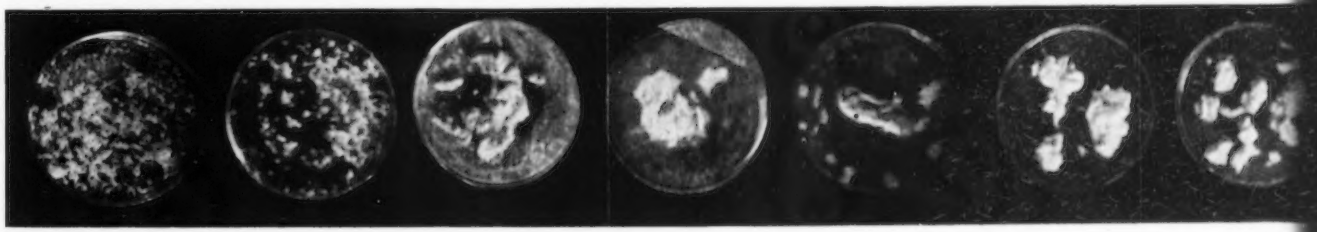
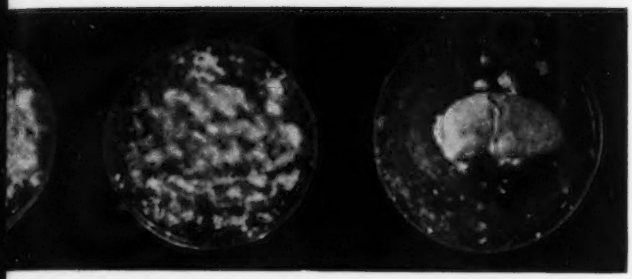
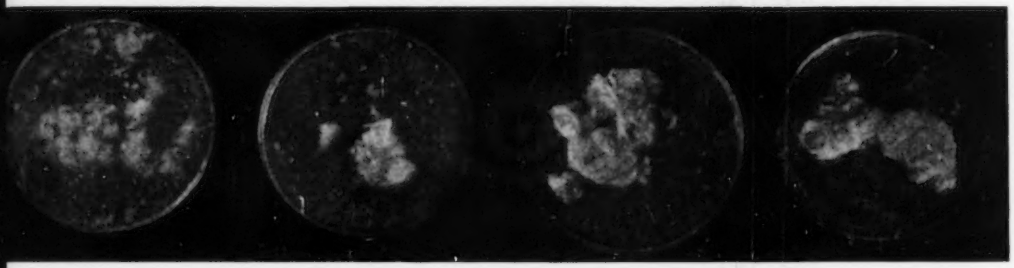


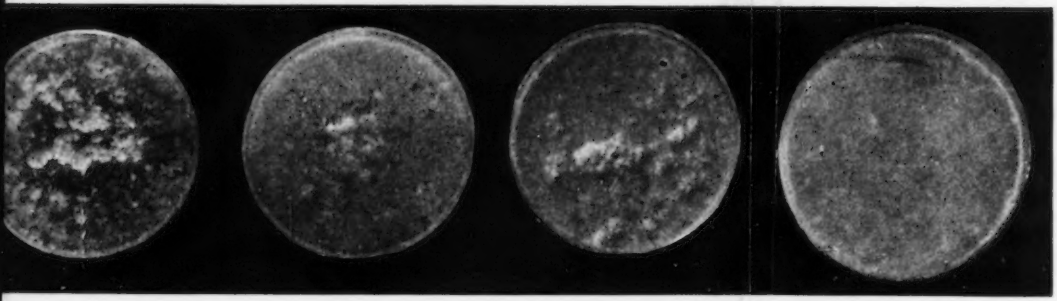
Fig. 3. Five hundred cubic centimeters raw skimmed milk at 37°C. Regurgitation after 0.5, 1, 2.5, 5, 10, 15, 25 and 60 minutes. (



17 seconds. Regurgitation after 1, 2, 3, 5, 10 and 25 minutes.



egurgitation after 0.5, 1, 2.5, 5, 10, 15, 30 and 40 minutes. (0.35 natural size.)



egurgitation after 1, 3, 7, 11, 15, 39, 55 and 60 minutes. Milk at 37°C. (0.37 natural size.)

tion after 0.5, 1, 2.5, 5, 10, 15, 25 and 60 minutes. (0.26 natural size.)

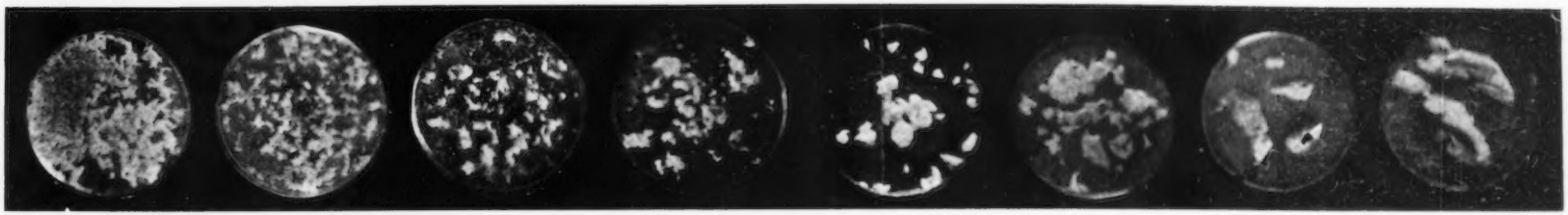


Fig. 4. Milk after water; 500 cc. water drunk in 5 minutes. After 3 minutes 500 cc. raw whole cows' milk at 37°C. drunk in 5 minutes. Regurgitation after 0.5, 1, 2.5, 5, 15, 20, 30 and 60 minutes. (0.26 natural size.)

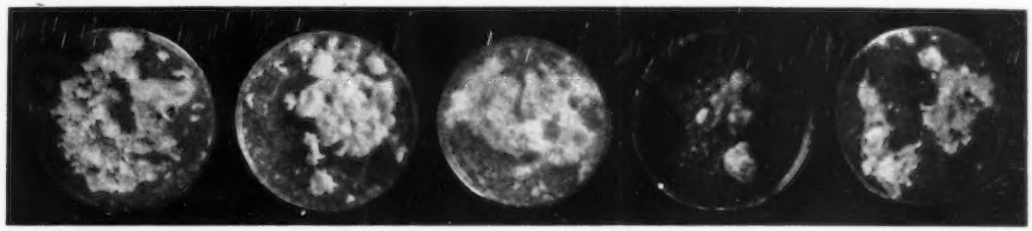


Fig. 5. Five hundred cubic centimeters raw whole milk with 2.5 gm. sodium bicarbonate. Regurgitation (early samples lost) after 20, 30, 40, 50 and 60 minutes. (0.27 natural size.)

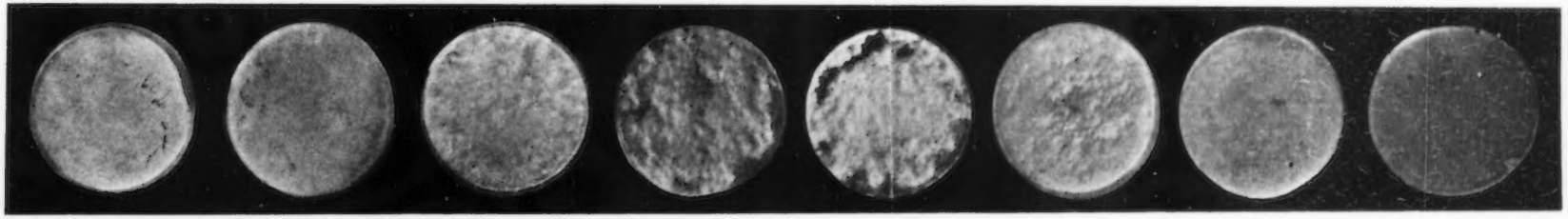


Fig. 6. Five hundred cubic centimeters cream (20 per cent milk fat) at 37°C. Drunk in 10 seconds. Regurgitation after 0.5, 2.5, 5, 10, 25, 60, 90 and 150 minutes. (0.27 natural size.)

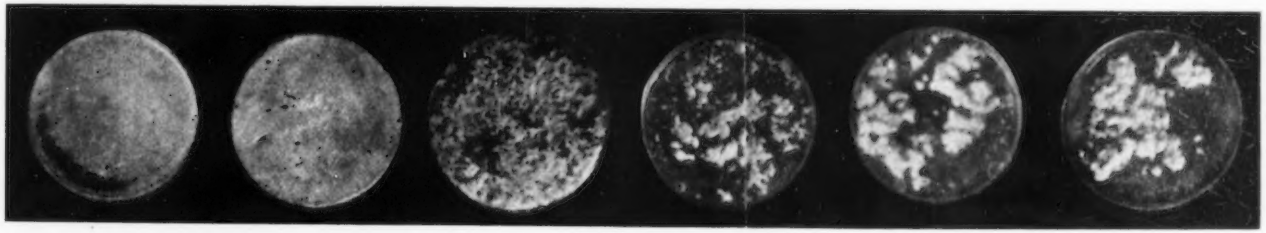


Fig. 7. Four hundred cubic centimeters boiled whole milk and 100 cc. raw whole milk. Drunk in 10 seconds. Regurgitation after 0.5, 2.5, 5, 10, 20 and 30 minutes. (0.27 natural size.)

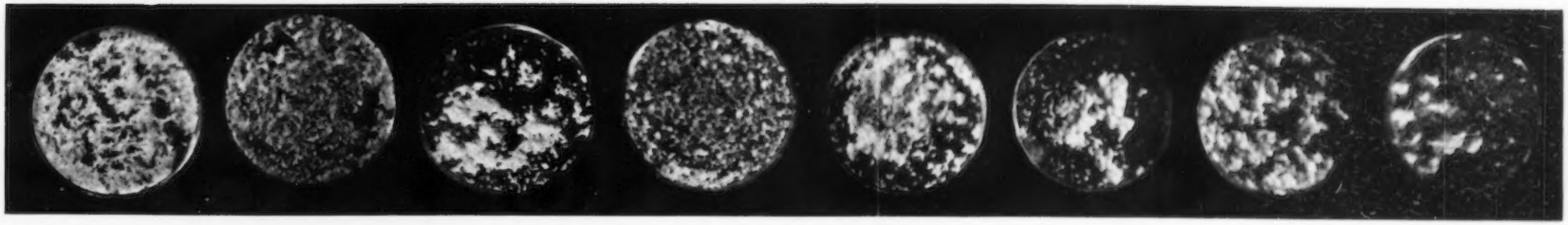


Fig. 8. Five hundred cubic centimeters skimmed milk boiled for 5 minutes. Taken at 37°C. in 10 seconds. Regurgitation after 0.5, 2.5, 5, 10, 20, 30, 40 and 50 minutes. (0.26 natural size.)

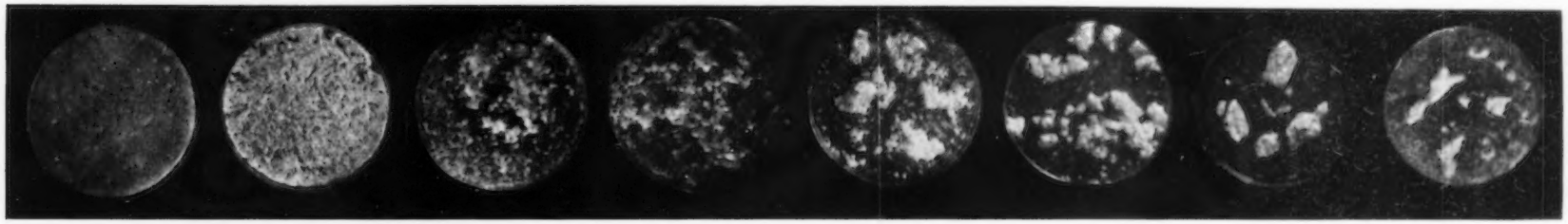


Fig. 9. Five hundred cubic centimeters pasteurized whole milk at 20°C. Drunk in 10 seconds. Regurgitation at 2.5, 5, 10, 15, 20, 30, 40 and 60 minutes. (0.27 natural size.)

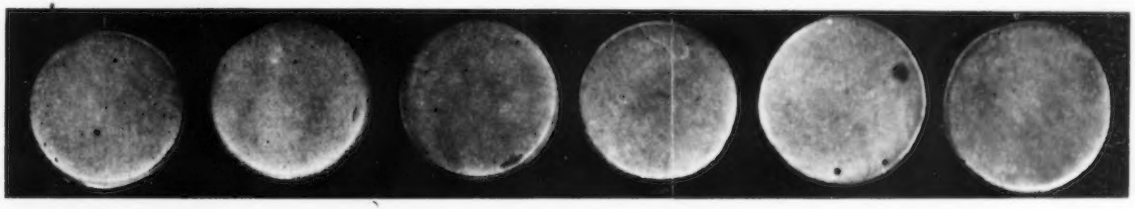


Fig. 10. Five hundred cubic centimeters cream (40 per cent butter fat). Drunk in 10 seconds. Regurgitation after 5, 15, 30, 60, 120 and 150 minutes. (0.25 natural size.)

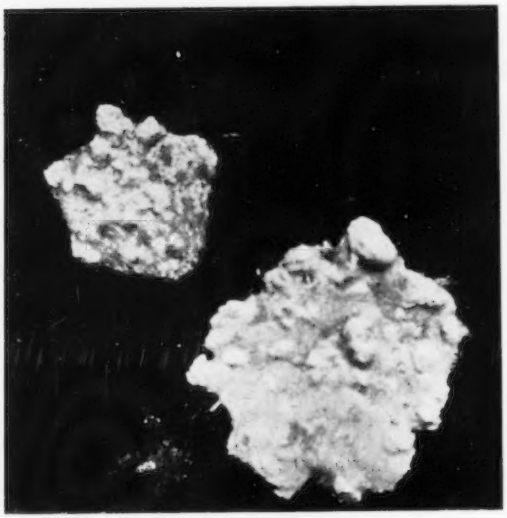


Fig. 11. Large curd mass = 500 cc. raw whole milk at 20°C. drunk in *10 minutes*. Small curd mass = 500 cc. raw whole milk at 20°C. drunk in *15 seconds*. (0.5 natural size.)

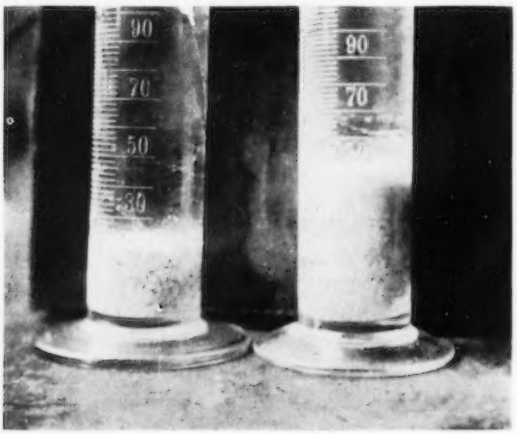


Fig. 12. Large curd mass = 500 cc. raw whole milk at 20°C. drunk in *10 minutes*. Small curd mass = 500 cc. raw whole milk at 20°C. drunk in *15 seconds*.

of water were drunk and by regurgitation 160 cc. of fluid with a few suspended curds were obtained.

Experiment 16. Milk drunk slowly.

Five hundred cubic centimeters of raw whole milk, 20°C., were drunk in nine and one-half minutes. One-half hour later the stomach was emptied completely by regurgitation.

Remarks. This experiment is a duplicate of no. 12 except that it followed the rapid drinking of milk instead of preceding it as in the case of experiment 12. The results were similar in the two cases.

DISCUSSION AND SUMMARY

Rapid drinking vs. slow drinking of milk. Milk drunk rapidly left the stomach sooner and produced a smaller curd mass than milk drunk slowly or "sipped." For photographs of curds which were regurgitated one-half hour after drinking 500 cc. of raw whole milk, eee figures 11 and 12.

Raw vs. boiled milk. Raw whole milk in the human stomach formed firm white curds which had "rubber-like" characteristics. The maximum curd formation took place at about one hour after the milk had entered the stomach. These large curds were apparently formed through the coalescing of numerous smaller curds. At this period curds as large as a man's thumb and weighing at least 15 grams may be formed.

Milk which had been boiled five minutes curded in an entirely different manner. In this case the curds were small, soft, flaky and of a yellow color. The largest curds were about the size of a small pea. These curds left the stomach sooner and were much more digestible than the tough curds resulting from the ingestion of the raw milk. Dietetically, therefore, boiled milk is to be preferred to the raw product. The boiling of milk for five minutes, as in our tests, did not materially alter its nutritive value or growth-promoting properties (2) but may have lowered the antiscorbutic value (3 and 4).

Skimmed vs. whole milk. Particularly tough, hard curds of large size were formed in the human stomach after the entrance of raw skimmed milk. These curds formed very quickly (one-half minute) and inside of five minutes they coalesced to form curds as large as a man's thumb. The skimmed milk curds, because of their firm, unyielding character, were more difficult to regurgitate than were the whole milk curds. The regurgitator also complained of a "heavy" feeling in his stomach soon after drinking the skimmed milk.

When the skimmed milk was boiled, the resulting curds were not as large and tough as were those of the raw skimmed milk nor as small and soft as were the curds of boiled whole milk. The largest curd (one hour) was about the size of a small kidney bean. Inasmuch as the boiled skimmed milk contained only about 0.5 per cent fat (sp. gr. 1034) whereas the boiled whole milk contained about 4 per cent fat it is apparent that the fat content is an important factor in determining the character of the curd in boiled milk. The curds of the boiled whole milk were digested more rapidly than were those of the boiled skimmed milk.

Influence of sodium bicarbonate on curd formation. The addition of two and one-half grams of sodium bicarbonate to 500 cc. of raw whole milk caused the formation of curds which were smaller and softer than those produced in similar milk in the absence of bicarbonate. There was a definite curd formation at five minutes although the stomach contents remained alkaline for thirty minutes. That the bicarbonate treatment was not as effective as was boiling in producing small soft curds was shown by the fact that the curds of the boiled whole milk were smaller and softer than were the curds in the milk after bicarbonate had been added. The boiled milk also left the stomach sooner than the bicarbonate milk.

Influence of milk fat on curd formation. As a general rule the character of the milk curds was influenced in a very direct way by the fat concentration, i.e., the more fat the less curd. Not only was there less total curd formation but the curds which were formed were smaller and softer the higher the fat concentration. In fact, when 40 per cent cream was drunk no curds were formed during the first half-hour and even then the largest curds were only about the size of the head of a pin and they did not coalesce to form larger curds as was the case in milk of low fat content.

The milks of high fat concentration were also very slow to leave the stomach. In fact two and one-half hours after 500 cc. of 40 per cent cream were ingested, at least 475 cc. of material were regurgitated from the stomach. These tests were rather distasteful to the regurgitator since the rich creams gave him a very uncomfortable, heavy feeling which bordered on nausea. There was a tendency for skimmed milk (0.5 per cent fat) to leave the stomach somewhat more slowly than the raw whole milk (4 per cent fat). We attribute this to the fact that the curds of the skimmed milk were especially tough and were slow to disintegrate under the influence of the gastric juice.

Influence of pasteurization on curd formation. Pasteurizing of milk is not nearly as effective as boiling in altering the type of curd formation. The curds from pasteurized milk were larger than those from boiled milk, but not so large as those from the raw whole milk. In short, the curds of the pasteurized milk were intermediate in character between the curds of raw and boiled milk, but had a tendency to resemble more closely the curds of raw milk. There was, however, less tendency for the pasteurized curds to coalesce with the formation of a large curd aggregate.

Influence of dilution on curd formation. The drinking of water before the milk was ingested had no material influence upon the curding tendencies of the milk, with the exception that the curds were somewhat softer and the maximum sized curds were smaller than when no water was ingested. It seems probable that the extra volume of fluid in the stomach prevented the smaller curds from coalescing with the formation of large masses.

Influence of temperature on curd formation. Milk curded very quickly in the stomach irrespective of the temperature. There was a slight slowing of the coagulation rate in the case of milks which were considerably below body temperature but the delay was not pronounced. The milks at 20°C. began to curd between one and two and one-half minutes whereas milks at 37°C. showed signs of curding at the one-half minute interval.

The curding of mixtures of boiled and raw milk. When a mixture composed of 80 per cent boiled whole milk and 20 per cent raw whole milk was drunk the resultant curds were very similar in character to those obtained from the ingestion of straight raw whole milk. Large curds were regurgitated at the twenty minute period, and the soft, yellow, fluffy curds typical of boiled milk were not in evidence.

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STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

I. The Colon. A Contribution to the One-Sided Permeability of the Intestinal Wall to Chlorides[1]

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The relative part played by the physical factors of diffusion, osmosis, filtration, imbibition, etc., and the so-called "physiological activity" of the living cells, in intestinal absorption, has received much attention in the past. It is still far from a settled problem.

The apparent property of the intestine of passing dissolved substances in but one direction has been especially difficult of interpretation, if one is to assume that physical factors are chiefly concerned in intestinal absorption. This seeming power of the intestine to direct the stream of dissolved substances through the intestinal wall in but one direction, has been a subject of much investigation.

Cohnheim (1), in experiments upon dogs with a Vella fistula of the small intestine, finds that sodium chloride passes into dextrose solutions in the isolated loop only in small amount, whether the dextrose solution is isontonic, hypertonic or hypotonic. The author attributes this small amount of sodium chloride to the secretion of the intestine. It is further shown that after a sufficient interval following death, or upon injury to the cells with sodium fluoride, arsenic, etc., the normal mechanism of one-sided permeability may be disturbed since, under these conditions, the sodium chloride content of the intestinal fluid increases above normal. This, he states, proves that the permeability, in but one direction, is a function of the living intestinal epithelium. In the peritoneal and pleural cavities the author finds that sodium chloride diffuses into sugar solutions in large amount.

[1] A preliminary report was made before the American Society of Biological Chemists, December, 1916; Journ. Biol. Chem., 1917, xxix, p. xii.

Waymouth-Reid (2) amplifies and confirms the work of Cohnheim on the one-sided permeability of the intestine. In experiments, using the mid-region of the ileum of dogs, the conclusion is reached that, except when the intestinal epithelium is injured, the small amount of sodium chloride entering the intestinal fluid originates in the succus entericus. Waymouth-Reid (3) believes that distilled water may injure the epithelium of the small intestine, but states that the lower regions are more resistant to this action than the upper. This one-sided permeability is accounted for by a "mechanism" which tends to drive the blood salts back as fast as they tend to diffuse into a bland solution lying in the gut. This same mechanism, the author states, forwards into the blood any salt entering the cells from the gut side. When sodium chloride is infused intravenously into dogs, Waymouth-Reid (3) finds that although the blood chlorides are increased, no increase in absorption of water is observed from glucose solutions in the ileum. Occasionally a decreased glucose absorption is found. Hence it is concluded that the uptake of water is not a simple function of the osmotic pressure of the sodium chloride of the blood, to which the normal epithelium is practically impermeable from the blood side to the gut side of the membrane, and since sodium chloride is the chief blood salt, there is probably no relationship to any other blood constituent. With Cohnheim the author believes that, in regard to water absorption, the osmotic factor is in relation to the action of the cells rather than to the blood.

Heidenhain (4) points out that from the ileum of dogs, solutions of sodium chloride of lower concentration than the blood (0.3 to 0.5 per cent) are absorbed, whereas if we were dealing with a purely physical phenomenon, sodium chloride should diffuse into the intestine. The conclusion is therefore drawn that while water absorption from these solutions is, in part at least, due to osmotic factors, the salt absorption is due to a "physiological activity" of the intestinal wall.

Hamburger (5), working with homogeneous membranes, the intestine of dead animals and with isolated tissues, believes to have demonstrated that the one-way permeability, together with other factors of absorption peculiar to the intestine, may be imitated in the artificial membranes and may proceed in an animal dead from one to twenty-four hours. He therefore concludes that the theory of a "physiological activity" is not justified. He would ascribe this activity to certain physical forces; molecular imbibition, intra-intestinal pressure and a suction induced by the blood stream. Therefore, the living cell is not involved.

Diena (6), using the permanently isolated colon of dogs, finds that there is a passage of substances from the interior of the organism into the intestine and vice versa. Diena reports that sodium chloride passes into strongly hypotonic solutions of this salt in the colon. This investigator further states that the quantity of such substances passing into the intestine is not related to the osmotic pressure of the liquid introduced.

The investigation to be presented was planned to determine whether the colon is impermeable to the chief blood salt, sodium chloride, in the direction of the blood into the intestinal lumen.

EXPERIMENTAL METHODS

The colon, in situ, of dogs was selected for these experiments. By the use of the colon there is assured a uniform region of the intestine. With the experimental procedure used, manipulation of the intestine is avoided thus making it possible to use the same loop repeatedly in each experiment. Furthermore, it was our hypothesis that the relatively slower absorption from the colon, than from the small intestine, might serve to allow us to study more accurately the progressive changes occurring in solutions introduced therein. E. Waymouth-Reid (7) states that absorption in the colon for all constituents of serum, for peptone and glucose, is far less per unit measured surface than in the middle region of the ileum. We are also not unaware of the possibility that qualitative as well as quantitative differences may be found, from the very nature of the structural differences of the colon and regions of the small intestine.

In a few of the experiments to be presented, decerebrate animals were employed. In others "pantopon" served as the anesthetic. Unless otherwise stated, however, paraldehyde was administered. The operation was performed under a light ether anesthesia in the two latter cases. Usually, one administration of the paraldehyde was sufficient for each animal. The dogs were kept warm throughout the period of experimentation. The animals were prepared as follows: A mid-line incision is made in the abdomen large enough to admit two fingers. The cecum is then brought into view and a strong ligature placed around the ileum at its junction with the cecum, care being exercised not to involve any blood vessels. A soft rubber tube is now inserted through an incision in the cecum and is there fixed in place. A second similar tube is inserted into the small intestine to avoid dis-

tention and pressure during the experiment, from the accumulation of fluids and gases. The abdominal wall is closed with a few stitches around the rubber tubes. A funnel is inserted into the colon tube for the introduction of fluids. At this stage the large intestine is washed free of feces with warm tap water and finally with warm physiological saline. A soft rubber tube similar to the first is now secured into the rectum of the dog by means of a pursestring suture.

Test fluids may thus be introduced into the upper colon tube and removed from the rectal tube with no manipulation of the intestine. Several tests to determine the accuracy of delivery of fluids, introduced and recovered in this way, show that we are dealing with a maximum error of 2 per cent. The solutions were always introduced warm. The colons of the experimental animals were examined at the conclusion of the experiments and were invariably of normal appearance.

Chloride determinations were made by the Volhard method or by the McLean and Van Slyke (8) method when a limited amount of fluid was available. The latter method was also used for blood chloride determinations. There was never enough protein present to interfere with the Volhard titration; this point was repeatedly controlled. All of the chloride figures are expressed as sodium chloride in the tables below. The depression of the freezing point was determined by means of the Bechmann apparatus. A constant depression below the freezing point was maintained, hence corrections for under-cooling are not made.

In the experiments given below the solutions were introduced in the intestine, allowed to remain for a given interval, removed, volume recorded, and specimens taken for chloride and Δ determinations. In some experiments the same solution was repeatedly removed, in others a different solution was introduced into the intestine.

EXPERIMENTAL.

It is found that when solutions of low concentrations of sodium chloride, or distilled water, are introduced into the colon, there is always a diminution of the volume, the extent of which is dependent upon the length of time the fluid is allowed to remain in the intestine. Upon a comparison of the total amount of sodium chloride introduced (in solutions below 0.16 per cent) with the amount recovered it is found that there is a gain of chlorides in the intestinal contents. As the concentration of the chlorides in the intestinal fluid rises, this gain drops to a low figure and finally shows a decrease in the chlorides,

with a greater deficit for each succeeding period thereafter. In other words, it is apparent that up to a certain point chlorides have entered the intestinal contents, followed by a reversal of the process, a passage of chlorides into the blood. The concentration and Δ of each successive solution removed shows a progressively increasing value. Typical protocols are given in table 1.

An exact determination of the concentration and Δ of the point, where the balance of chlorides ceases to be in favor of the gut contents and begins to exhibit a deficit, presents difficulties. It may be either in the last period, showing an excess of chlorides in the intestinal fluid over the amount initially in the solution, or it may equally well be in the first period in which there is a loss of chlorides from the colon contents. Therefore, it can only be said with certainty that the reversing of the phenomenon takes place within a certain range of the concentration and Δ. This range has been placed between the average of the last period of gain of chlorides in the gut and the average of the first period of loss of chlorides from the intestinal fluid. The range is compiled in table 2. An average value is also given of the initial and final concentrations of these two periods. The variation of this average figure, from the individual figures of each experiment is, in every case, less than the physiological variation in different animals.

In order to determine whether this phenomenon is related to the concentration of blood chlorides, observations similar to those above were made before and during intravenous infusion of sodium chloride. Since the kidneys were intact sodium chloride was infused in rather large amounts, slowly, over a long period of time, to insure a definite disturbance of the osmotic equilibrium in the body. Blood chloride determinations show that in each case the blood content of chlorides is appreciably increased. The results are presented in table 3. Under these conditions there is, in each experiment, an increase in the range of concentration of chlorides in which there ceases to be a gain in the gut and a beginning loss of chlorides. These increased values are beyond the physiological variation in an individual animal, hence, we believe, are significant under the experimental conditions. In experiment 69 the range of the control period is from 0.11 to 0.15 per cent, with an average of 0.13 per cent. During the infusion period the values are from 0.24 per cent to 0.29 per cent, with an average of 0.27 per cent. The range of the Δ is also correspondingly higher. Experiment 87 shows a similar behavior. These results, under the conditions described, show that with a large increase of blood chloride content

TABLE 1

The reversible diffusion of chlorides through the intestinal wall

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl: + GAIN, − LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED
	minutes	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*		
37	30	98	76	0 04	0 11	+44		0.119
	30	90	66	0.05	0.10	+21	0.037	0.109
	30	93	72	0.07	0.12	+21	0.047	0.109
	30	93	72	0.09	0.14	+17	0.065	0.121
	30	92	72	0.11	0.15	+ 7	0.078	0.123
	30	92	75	0.12	0.17	+17	0.085	0.145
	30	92	72	0.14	0.18	0	0.102	0.142
	30	93	76	0.16	0.19	−·4	0.115	0.145
38	30	·96	80	0.12	0.15	+12	0.082	0.126
	30	96	78	0.14	0.17	− 2	0.100	0.141
	30	95	77	0.16	0.18	−14	0.111	0.150
	30	96	75	0.18	0.19	−30	0.120	0.157
	30	95	71	0.19	0.20	−39	0.134	0.160
84	30	100	79.5	0.11	0.17	+25	0.094	0.164
	30	100	81	0.13	0.18	+16	0.102	0.161
	30	100	77.5	0.15	0.18	−10	0.112	0.173
	30	100	79.5	0.17	0.19	−19	0.124	0.171
	30	100	77	0.19	0.21	−28	0.131	0.166
87	30	100	79	0.10	0.17	+34	0.061	0.138
	30	100	75	0.11	0.16	+ 9	0.061	0.135
	30	100	76.5	0.13	0.17	0	0.081	0.131
	30	100	75	0.15	0.18	−15	0.091	0.136
	30	100	75	0.17	0.20	−20	0.116	0.162
	30	100	74	0.19	0.21	−35	0.123	0.161

In the following experiments the solution was removed, specimen taken for analysis and remainder returned:

Pantopon	30	100	68		0.07	+48		
59	30	48	37	0.07	0.11	+ 7		
	20	32	21	0.11	0.13	− 7		0.184
35	60	127	75	0 08	0.17	+26		
	60	55	33	0.17	0.18	−34		
	60	78	40	0.17	0.21	−49		
Decerebrate	60	90	75	0 06	0.15	+59		
78	60	64.5	49	0.15	0.19	+ 4		
	60	39	25	0.19	0.22	−19		
85	30	140	110	0.08	0.14	+42		
	30	105	82	0.14	0.18	+ 1		
	30	77	57	0.18	0.21	−19		0.205
Pantopon	10	132	120	0.10	0.12	+12	0.072	0.102
61	10	100	94	0 12	0.14	+12	0.102	
	10	84	79	0.14	0.15	+ 1		0.134
	10	58	54	0.15	0.17	+ 3	0.134	0.142
	10	34	29	0.17	0.19	− 3	0.142	0.174

there is a significant increase in the range and average concentration at which the balance of chloride in the colon contents ceases to be in favor of the intestine. It may be added that in experiment 86, where the volume introduced and the time of sojourn were constant, there is no indication of an increased water loss from the intestinal fluid.

The excess of chloride which has been demonstrated to be present in solutions of very low concentration in the colon, may have its origin

TABLE 2

Chloride threshold. Summary of data

EXPERIMENT NUMBER	AVERAGE CONCENTRATION OF NaCl		AVERAGE CONCENTRATION	AVERAGE Δ		AVERAGE Δ	REMARKS
	Last period of gain	First period of loss		Last period of gain	First period of loss		
	per cent	*per cent*	*per cent*				
35	0.13	0.18	0.16				Same solution repeatedly returned
37	0.16	0.18	0.17	0.122	0.130	0.126	New solution introduced each time
38	0.14	0.16	0.15	0.104	0.121	0.113	New solution introduced each time
49	0.10	0.13	0.12				Same solution repeatedly returned
59(a)	0.09	0.12	0.11				Same solution repeatedly returned
(b)	0.11	0.15	0.13				Same solution repeatedly returned
61	0.16	0.18	0.17	0.138	0.158	0.148	Same solution repeatedly returned
69(a)	0.20	0.23	0.22				Same solution repeatedly returned
(b)	0.11	0.15	0.13	0.100	0.143	0.122	Same solution repeatedly returned
78(a)	0.17	0.21	0.19				Same solution repeatedly returned
(b)	0.11	0.13	0.12	0.114	0.134	0.124	Same solution repeatedly returned
84	0.16	0.17	0.17	0.132	0.143	0.138	New solution introduced each time
85(a)	0.26	0.27	0.27	0.194	0.199	0.197	New solution introduced each time
(b)	0.16	0.20	0.18				Same solution repeatedly returned
87	0.16	0.17	0.17	0.098	0.106	0.102	New solution introduced each time
Average.....			0.16			0.134	

in the walls or cells of the intestine, and therefore, represent only a local diffusion from the intestinal wall. If this were the case, it might be expected that when the process has proceeded to a certain point, it would of necessity come to a standstill. Hence, if repeatedly renewed portions of distilled water were brought into contact with the colon, on the above supposition, decreasing amounts of chloride should pass into the fluid at each successive period of observation, and should eventually reach a very low value.

TABLE 3

Effect of increased blood chlorides upon the reversible diffusion of chlorides through the intestinal wall

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl: ! GAIN + LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	REMARKS
				Control period					
	mins.	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*			
Pantopon 61	10	132	120	0.10	0.12	+12	0.072	0.102	
	10	100	94	0.12	0.14	+12	0.102		
	10	84	79	0.14	0.15	+ 1		0.134	
	10	59	54	0.15	0.17	+ 3	0.134	0.142	
	10	34	29	0.17	0.19	− 3	0.142	0.174	Blood NaCl = 0.61 per cent
				Infused 96 cc. of 10 per cent NaCl intravenously over period of two hours					
	10	141	131	0.10	0.13	+29	0.087		
	10	121	112	0.13	0.15	+11		0.127	
	10	92	83	0.15	0.19	+20	0.127	0.142	
	10	65	60	0.19	0.21	+ 3	0.142		Blood NaCl = 1.1 per cent
	10	50	44	0.21	0.24	0		0.182	
				Control period					
69	15	120	100	0.08	0.13	+34	0.074	0.126	Blood NaCl = 0.62 per cent
	20	80	62	0.13	0.16	− 5	0.126	0.160	
	15	45	31	0.16	0.19	−13	0.160	0.202	
				Infused slowly 100 cc. of 10 per cent NaCl intravenously during two hours					
	15	118	97	0.09	0.16	+49	0.075	0.128	
	15	80	68	0.16	0.21	+15	0.128	0.185	
	15	50	40	0.21	0.27	+ 3	0.185	0.258	Blood NaCl = 0.82 per cent
	15	24	15	0.27	0.30	−20		Average 2 periods	
				Control period					
87	30	100	79	0.10	0.17	+34	0.061	0.138	
	30	100	75	0.11	0.16	+ 9	0.061	0.135	
	30	100	76.5	0.13	0.17	0	0.081	0.131	
	30	100	75	0.15	0.18	−15	0.091	0.136	
	30	100	75	0.17	0.20	−20	0.116	0.162	
	30	100	74	0.19	0.21	−25	0.123	0.161	
	30	100	74	0.22	0.23	−50	0.136	0.191	
	30	100	74	0.27	0.28	−63	0.190	0.218	
				Infused 150 cc. of 10 per cent NaCl intravenously during two hours					
	30	100	70	0.19	0.29	+13			
	30	100	75	0.25	0.31	−17			
	30	100	80	0.27	0.33	− 6			

TABLE 4

The diffusion of chlorides into distilled water in colon

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl RECOVERED	GAIN IN NaCl TO GUT CONTENTS	Δ FLUID RECOVERED	REMARKS
	mins.	*cc.*	*cc.*	*per cent*	*mgm.*		
37	30	100	89	0.18	160	0.189	
	30	100	84	0.15	126	0.162	
	30	100	89	0.12	107	0.115	
	30	100	79	0.11	87	0.110	Total NaCl = 0.480 gram
38	30	100	83	0.10	82	0.103	
	30	100	83	0.07	58	0.080	
	30	100	80	0.08	64	0.084	
	30	100	80	0.06	48	0.065	Total NaCl = 0.252 gram
54	30	93	77	0 08	62		Thymol added to contents in colon
	30	100	72	0.07	50		for other purposes
	30	100	77	0.06	46		
	30	100	70	0.06	42		
	30	100	71	0.05	36		
	30	100	73	0.05	37		
	30	100	59	0.09	53		Total NaCl = 0.326 gram
55	60	96	65	0.17	110		
	60	100	62	0.13	81		
	60	100	56	0.10	56		
	60	100	56	0.08	45		Total NaCl = 0.292 gram
42	60	100	70	0.19	136	0.219	At beginning of experiment
							Blood Δ = 0.604
							Blood NaCl = 0.62 per cent
	60	100	72	0.14	101	0.160	
	60	100	69	0.10	69	0.211	
	60	100	65	0.10	65	0.120	
	60	100	69	0.10	69	0.110	At end of experiment
							Blood Δ = 0.554
	60	100	68	0.10	68	0.121	Blood NaCl = 0.54 per cent
	60	100	71	0.08	58	0.099	Total NaCl = 0.566 gram
		Effect of increased blood chlorides					
28	60	100	64	0.08	51		At beginning of experiment
	60	100	59	0.07	41		Blood NaCl = 0.59 per cent
	60	100	63	0.07	44		
	60	100	60	0.07	42		
		Infused 158 cc. of 10 per cent NaCl during 1 hour 20 minutes					
	60	100	65	0.08	52		
	60	100	66	0.14	92		
							At end of experiment
	60	100	65	0.13	85		Blood NaCl = 0.88 per cent
							Total NaCl = 0.407 gram

Experiments designed to test this point are given in table 4. There is no evidence from these and other figures which are available, that we are dealing with a very limited source of chlorides. In the first periods the values may be somewhat higher than in the succeeding periods; however, this excess might very well arise from the fluids bathing the intestine. The subsequent values are significantly high. The total amount of chlorides withdrawn in each experiment is also calculated, and give values from 0.250 to 0.590 gram of sodium chloride. The total amount of chlorides present in the intestine is a function of the time which the fluid is allowed to remain in the gut. Comparisons of half and one hour experiments are made. That the continued withdrawal of chlorides through the intestine may affect the blood

TABLE 5

Diffusion of chlorides into distilled water in colon of dead animal

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME (−LOSS)	NaCl RECOVERED	CHANGE NaCl: −LOSS+GAIN	REMARKS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*mgm.*	
31	30	90	87	−3	0.02	+17	Intestine in situ
	30	90	88	−2	0.02	+18	
	30	90	89	−1	0.02	+19	
	30	90	90	0	0.01	+ 9	
32	60	90	90	0	0.09	+81	Intestine removed from body
	60	90	87	−3	0.04	+35	
	75	90	87	−3	0.02	+17	

chlorides and the blood Δ is shown in experiment 42. A withdrawal of 0.556 gram of sodium chloride from this animal, resulted in a decrease of blood chlorides as sodium chloride from 0.62 per cent to 0.54 per cent, with a decrease in the blood Δ from 0.604 to 0.554.

Experiment 28, at the end of table 4, demonstrates the effect of intravenous infusion of sodium chloride upon the passage of chlorides into distilled water in the colon. A graphic representation is also given in figure 1, where the total amount of chlorides (as sodium chloride) diffusing into the intestine is represented. The time and volume of fluid are constant in this experiment. It is quite evident that an increase of blood chlorides, under these conditions, causes an increase in the concentration and total amount of chlorides in the intestinal

fluid. An increased absorption of water from the intestine is not demonstrable; the values before and during infusion are of the same order.

In this connection it seemed of interest to determine the changes occurring in distilled water in the intestine of dead animals. In experiment 31, (table 4) the intestine was allowed to remain in situ; in experi-

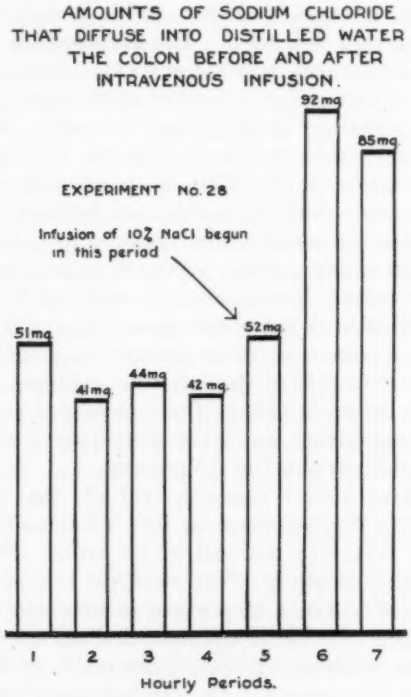

Fig. 1

ment 32 it was removed from the body and kept moist throughout the experiment.

In contrast to the former experiments, the distilled water in the intestine of a dead animal shows little or no tendency to disappear. Furthermore, the concentration of chlorides and the total amount present is much less than in the experiments on living animals, and decreases rapidly with each successive period. The total amount of

chlorides withdrawn might well have its origin in the walls and cells of the intestine. This, also, points to the fact that the ultimate origin of the chlorides, withdrawn in our experiments on living animals, is the blood.

DISCUSSION

The experimental work presented herewith demonstrates that up to the point where the intestinal contents reach a certain concentration, chlorides may pass into the colon. The approximate value of this concentration is 0.16 per cent, with a Δ of 0.134. It is evident that we are dealing here with a "factor of safety" in the form of a threshold value which has its analogy in the kidney threshold. It is the result of a mechanism which tends to prevent the loss of large amounts of body salt through the intestine. That this mechanism stands in relation to the blood content of salt, at least in part, has been demonstrated. A comparison of these results with those obtained by Cohnheim (1) and Waymouth-Reid (2) is not possible, inasmuch as their work was done upon the small intestine. Nevertheless, it is evident from the results presented that the colon is not characterized by a strictly one-sided permeability. The failure of these authors to demonstrate large amounts of sodium chloride in the intestinal contents may have its explanation in our threshold value. Our results also show why, as in Heidenhain's (4) experiments, an excess of sodium chloride cannot be demonstrated in solutions of 0.3 to 0.5 per cent.

We cannot believe with Waymouth-Reid (2) that this influx of sodium chloride into distilled water or into solutions below 0.16 per cent is due to an injury to the wall of the colon. Physiologically, there is no evidence of injury. The ultimate behavior of distilled water, or of a 0.2 per cent or a 0.5 per cent sodium chloride solution in the colon, is the same. Water is absorbed, and the concentration and Δ rise with all three solutions; the only difference to be found is in the fact that chlorides may pass into the distilled water, whereas at the higher concentrations there is a loss of chloride. We have repeatedly, in the course of our work, introduced distilled water into the colon, and subsequently tested the behavior of other solutions. A qualitative or quantitative difference in the behavior after distilled water or physiological saline has never been noted.

Our results are in accord with those of Waymouth-Reid (2) that there is not an increased water absorption from the intestine when the blood chloride content is raised by intravenous infusion of sodium

chloride. However, we have shown that an increased amount of chlorides passes into distilled water, with a rise in the threshold, under these conditions.

Two protocols in the paper by Diena (6) show a positive balance of sodium chloride in the intestine, after a solution of 0.19 per cent sodium chloride is allowed to remain in the colon for two hours. The final concentrations are, respectively, 0.4 and 0.475 per cent. In none of our experiments, under the most extreme conditions, (intraveous infusion of sodium chloride), have we ever found a positive balance of chlorides at a final concentration of 0.4 per cent. We wish to suggest that this difference is due to a difference in the experimental methods employed. At the end of the period of observation Diena washes the intestine with distilled water (270 to 300 cc.). The chlorides thus withdrawn are added to the total chlorides recovered. Our experiments show that chlorides pass rapidly into distilled water in the colon. Experiments have been performed in which distilled water was merely passed through the intestine, and we find that considerable amounts of chlorides may be thus abstracted. The chlorides of the wash fluid, in each of Diena's experiments, are greater than the excess of chlorides found by him. Hence, disregarding the amount of chlorides recovered in this fashion, his experiments show a loss of chlorides from the colon. We are in accord with Diena's conclusion that chlorides pass into strongly hypotonic solutions in the colon, but cannot agree that this occurs at the sodium chloride concentrations of the final fluids withdrawn by him.

CONCLUSIONS

1. The colon is not characterized by a strictly one-sided permeability.
2. There is a threshold of the colon, below which chlorides diffuse from the blood stream into the intestinal contents.
3. The blood content of chlorides is a factor in determining the height of this threshold.
4. The chlorides diffusing into distilled water or very low concentrations of sodium chloride in the colon have their origin in the blood.

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STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

II. The Colon. On the Passage of Fluid in Two Directions through the Intestinal Wall[1]

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It has been shown (1) that there is a threshold value above which loss of chlorides from the blood into the intestinal fluid is inhibited, under ordinary conditions. It seemed of interest, therefore, to determine whether a similar "factor of safety" controlled water loss from the blood into the intestine.

Heidenhain (2) finds that water may be absorbed from solutions of sodium chloride of various concentrations in the small intestine, that is from a 1.46 per cent as well as from a 2.0 per cent solution. He considers that the fluid absorption in these cases is a function of the "physiological activity" of the intestinal epithelium. Since the osmotic pressure of the solution is greater than that of the blood the osmotic factors are not concerned in the process. Although the "physiological activity" is capable of varying in value, the author states that the border-line concentration at which it is overcome is at 2.0 per cent of sodium chloride. Above this point osmotic pressure factors come into play and fluid passes into the intestine, but the author states that solutions of sodium chloride above 2.0 per cent may exert an injurious effect upon the intestinal epithelium and thus modify the absorption phenomenon.

On the basis of investigations upon the isolated small intestine of cats, rabbits and dogs, Cohnheim (3) believes to have demonstrated an active transport of fluid, inasmuch as there is a loss of fluid from the intestine with no increase of weight of the intestine. This transport in

[1] A preliminary report was made before The American Society of Biological Chemists, December, 1916. Journ. Biol. Chem., 1917, xxix, p. xii.

one direction, toward the blood, has its origin in the cells of the intestinal wall.

Waymouth-Reid (4) confirms the observation of Cohnheim that a secretory transport of fluid is capable of demonstration in the isolated small intestine of cats. This takes place in all instances from the intestinal lumen.

We well realize that the behavior of the fluid of solutions introduced into the intestine depends upon the nature of the solute, as Wallace and Cushny (5) and Höber (6) have shown. The intestinal wall is readily permeable to sodium chloride, hence the results of the present investigation would apply only to salts of this class.

The experimental methods are similar in all respects to those used in the previous paper (1). Since the volume of fluid remains sufficiently large, the same solutions are repeatedly returned in all of the experiments given below.

EXPERIMENTAL

In the typical protocols, given in table 1, it is shown that when solutions of sodium chloride (above 1.2 per cent) of osmotic pressure sufficiently higher than the blood, are introduced into the colon of dogs, fluid passes into the intestinal contents while chlorides pass into the blood. The effect is a rapid reduction in the sodium chloride concentration and osmotic pressure of the intestinal contents. Figure 1 gives a graphic representation of these volume and concentration changes in a typical experiment.

In determining the concentration of sodium chloride and the Δ at which the fluid balance ceases to be positive in the intestine, and fluid begins to leave, we are confronted with the same difficulties as in the determination of the salt threshold in our previous paper. The turning point may be either in the last period which shows a gain of fluid to the gut, or in the first period which exhibits a loss of fluid.

Hence in table 2 has been compiled the average concentration of sodium chloride in each of these periods. This gives a range between which the point must lie. An average of the range of each experiment has also been noted. This gives an approximate value for the concentration of chlorides, at the point where the fluid content of the colon turns from a positive to a negative balance. The average Δ has been computed similarly, thus an approximate value of sodium chloride concentration of 1.21 per cent with a Δ of 0.786 is obtained. It will be seen that the variations in the individual experiments are not great,

TABLE 1

The reversible diffusion of fluid through the intestinal wall

EXPERIMENT NO.	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME − LOSS, + GAIN	NaCl INTRODUCED	NaCl RECOVERED	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	CHANGE NaCl IN GUT
	min.	*cc.*	*cc.*		*per cent*	*per cent*			*mgm.*
17	60	28	43	+15	4.22	1.56			−511
	60	42	47	+ 5	1.56	1.06			−157
	60	46	36	−10	1.06	0.81			−196
	60	35	17	−18	0.81	0.64			−175
29a	30	100	113	+13	1.99	1.55			−240
	30	109	118	+ 9	1.55	1.33			−120
	30	114	112	− 2	1.35	1.15			−228
	30	108	101	− 7	1.15	1.05			−182
29b	30	95	102	+ 7	1.45	1.19		0.821	−164
	30	88	85	− 3	1.19	1.07	0.821	0.721	−138
	30	71	65	− 6	1.07	0.96	0.721	0.662	−136
	30	51	41	−10	0.96	0.90	0.662	0.670	−121
30	30	86	103	+17	1.57	1.35	0.987	0.878	
	30	89	94	+ 5	1.35	1.20	0.878	0.795	− 74
	30	80	78	− 2	1.20	1.07	0.795	0.702	−125
	30	64	63	− 1	1.07	1.00	0.702	0.671	− 55
	30	49	41	− 8	1.00	0.90	0.671	0.626	−130
33	30	131	139	+ 8	1.64	1.39	1.009	0.901	−216
	30	125	134	+ 9	1.39	1.23	0.901	0.820	− 89
	30	120	125	+ 5	1.23	1.13	0.820	0.745	− 64
	30	111	109	− 2	1.13	1.00	0.745	0.680	−164
	30	95	86	− 9	1.00	0.94	0.680	0.674	−142
	30	72	64	− 8	0.94	0.88	0.674	0.660	−114
	30	50	40	−10	0.88	0.84	0.660	0.630	−104
34	30	134	134	0	1.65	1.48	1.005	0.930	−228
	30	120	122	+ 2	1.48	1.33	0.930	0.838	−153
	30	108	118	+10	1.33	1.19	0.838	0.771	− 32
	30	104	102	− 2	1.19	0.07	0.771	0.702	−146
	30	88	83	− 5	1 07	0.97	0.702	0.676	−137
	30	69	62	− 7	0.97	0.89			−118
	30	48	41	− 7	0.89	0.85		0.603	− 79
Decerebrate	10	100	112	+12	1.90		1.200		
60	15	112	116	+ 4		1.48		0.934	−184
	15	100	102	+ 2	1.48		0.934		
	15	102	102	0		1.24		0.838	−215
	15	85	80	− 5	1.24	1.20	0.838	0.827	− 94
	15	65	58	− 7	1.20				
	15	58	53	− 5		1.09			−202
	20	49	38	−11	1.09	1.04		0.743	−139

TABLE 1—*Continued*

EXPERIMENT NO.	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME − LOSS, + GAIN	NaCl INTRODUCED	NaCl RECOVERED	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	CHANGE NaCl IN GUT
	min.	*cc.*	*cc.*		*per cent*	*per cent*			*mgm.*
Decerebrate	15	100	107	+ 7	1.96		1.96		
74	15	107	115	+ 8		1.48		0.917	−260
	15	101	103	+ 2	1.48		0.917		
	15	103	104	+ 1		1.21		0.781	−236
	15	90	88	− 2	1.21	1.11	0.781	0.737	−112
	15	75	70	− 5	1.11	1.05	0.737	0.686	− 98
	30	57	50	− 7	1.05	0.96	0.686	0.646	−119
Effect of NaCl infusion (intravenously) upon "crest" of volume curve									
65	15	146	149	+ 3	1.91		1.180		
	15	149	158	+ 9		1.64		1.159	−197
	15	144	151	+ 7	1.64		1.159		
	15	151	157	+ 6		1.47		0.949	− 54
	15	143	147	+ 4	1.47	1.40	0.949	0.921	− 44
	15	133	139	+ 6	1.40	1.30	0.921	0.851	− 55
	15	125	128	+ 3	1.30	1.24	0.851	0.821	− 38
	15	108	110	+ 2	1.24	1.17	0.821	0.774	− 52
	15	90	92	+ 2	1.17	1.11	0.774	0.741	− 32
	15	72	71	− 1	1.11	1.03	0.741	0.697	− 68
	15	51	50	− 1	1.03	0.97	0.697		− 40
	35	46	42	− 4	0.97	0.85		0.610	− 89
Infused intravenously 116 cc. of 10 per cent NaCl over period of four hours									
	30	90	89	− 1	1.46	1.41			
	30	85	81	− 4	1.41	1.38		0.892	
Pantopon	15	90	90	0	1.44		0.955		
59	15	90	94	+ 4		1.10		0.744	−262
	15	80	75	− 5	1.10	0.98	0.744	0.705	−145
	20	60	54	− 6	0.98	0.87	0.705	0.656	−118

and differ but slightly from the average. This is the more striking when one considers that physiological variations as well as experimental errors are involved.

In table 1 has been added a single protocol showing the effect of increased blood chloride content upon this phenomenon. It will be seen that, whereas in the normal period there is a gain of fluid (4 cc.) to the gut at a concentration of sodium chloride of 1.47 to 1.40 per cent, at a corresponding concentration, during infusion, there is prac-

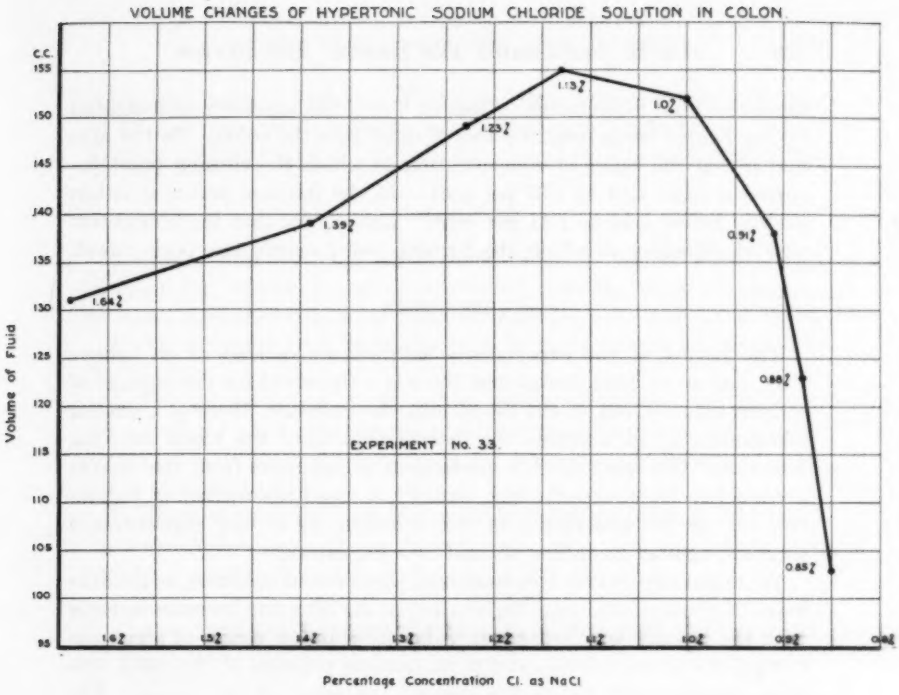

Fig. 1

TABLE 2

Fluid threshold. Summary of data

EXPERIMENT NUMBER	AVERAGE CONCENTRATION OF LAST AND FIRST PERIODS RESPECTIVELY, IN WHICH GUT CONTENTS SHOW		AVERAGE CONCENTRATION	AVERAGE Δ OF LAST AND FIRST PERIODS RESPECTIVELY, IN WHICH GUT CONTENTS SHOW		AVERAGE Δ
	Gain in volume	Loss in volume		Gain in volume	Loss in volume	
	per cent	*per cent*	*per cent*	Δ	Δ	Δ
17	1.31	0.94	1.13	—	—	—
29(a)	1.44	1.24	1.34			
29(b)	1.32	1.13	1.23			
30	1.28	1.14	1.21	0.839	0.749	0.793
33	1.18	1.07	1.13	0.783	0.713	0.748
34	1.26	1.13	1.20	0.805	0.737	0.771
59	1.27	1.04	1.16	0.850	0.725	0.788
60	1.36	1.22	1.29	0.886	0.833	0.860
61	1.14	1.07	1.11	0.758	0.719	0.739
74	1.35	1.17	1.26	0.849	0.759	0.804
Average.....			1.21			0.786

tically no fluid interchange (a loss of 1 cc.), the tendency as indicated by the figures being toward a loss of fluid from the colon. In the normal period the range of concentration in which the turning point occurred is from 1.14 to 1.07 per cent. In the infusion period it is but slightly below 1.46 to 1.41 per cent. This shows that the concentration of chlorides at which the turning point occurs has been raised.

DISCUSSION

The results of the experiments detailed above lead us to believe that, just as we have found that there is a threshold for the passage of sodium chloride out of the blood into the intestine, there is a similar mechanism which controls the loss of fluid from the blood into the intestine. One part of this mechanism is apparent from the results obtained in these experiments, namely, a rapid absorption of sodium chloride or the absorption of the chlorides in a very concentrated solution, against an inflow of fluid into the intestine.

We might expect that this combined effect would continue, as Heidenhain (2) pointed out, until the solution in the intestine becomes isotonic with the blood. On the contrary, the water inflow ceases at a concentration of sodium chloride and at an osmotic pressure of the colon contents considerably above the osmotic pressure of the blood. The chloride absorption continues rapidly to reduce the osmotic pressure of the solution in the colon. Hence, although we are in agreement with Heidenhain's (2) conclusion that solutions of sodium chloride of higher osmotic pressure than the blood may exhibit fluid absorption in the intestine, the concentration at which fluid passes into the colon contents (approximately 1.21 per cent) is much lower than that found by Heidenhain in the small intestine, namely 2.0 per cent.

It will, moreover, be noted that in our experiments the same results are obtained with a sodium chloride concentration of 4.22 per cent, experiment 17, as with a solution of 1.45 per cent, experiment 29b. The difference in concentration of these two solutions is so great that we cannot but believe that if, as Heidenhain (2) suggested, strong solutions of sodium chloride injure the intestinal wall, there would be an evident difference of effect in these two experiments.

Cohnheim (3) and Waymouth-Reid (4) have attributed the fluid absorption in the intestine to an "active transport," which is a function of the epithelial cells of the intestine. We have shown that an increase of blood chlorides, by intravenous infusion of sodium chloride,

raises the water threshold. Therefore, this "active transport" may be altered from the blood side, hence we cannot affirm the view held by these authors that it is entirely a function of the intestinal epithelium.

CONCLUSIONS

1. Hypertonic solutions of sodium chloride which readily pass through the intestinal wall, attract fluid into the intestine above a certain threshold value, approximately 1.20 per cent with a Δ of 0.786.

2. At the same time chlorides pass in concentrated solutions into the blood.

3. The concentration of the solute (sodium chloride) and the Δ at the threshold point, that is the point at which the fluid balance just ceases to be in favor of the colon, shows a striking constancy in a long series of experiments.

4. There is evidence that increased blood chlorides may effect this threshold value.

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STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

III. The Colon. The Osmotic Pressure Equilibrium between the Intestinal Contents and the Blood

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A further link in the comparison of the passage of solute and solvent through the intestine, with diffusion through homogeneous membranes is a study of the partial pressure equilibrium of diffusible dissolved substances and the total osmotic equilibrium. An investigation of these points is presented in this paper.

Gumilewsky (1), using animals with a Thiry-Vella fistula of the small intestine, finds that from solutions of sodium chloride of a concentration of 0.6 per cent the water and salt are absorbed at the same rate, hence the concentration is not changed. From solutions of 0.25 per cent sodium chloride, the author finds that water is absorbed faster than the salt, whereas the process is reversed from 1.0 per cent solutions.

Heidenhain (2) makes the same observation in regard to solutions of 0.6 per cent sodium chloride.

Waymouth-Reid (3) finds that glucose, and mixtures of glucose and sodium chloride, can be chosen which neither increase nor decrease in concentration.

Höber (4) states that when solutions, isotonic with the blood serum, are subjected to intestinal absorption, the osmotic pressure of the solution during absorption does not remain unchanged but is occasionally raised.

Diena (5), using the permanently isolated colon of dogs, investigated the behavior of solutions of sodium chloride at various concentrations. This author finds a large quantity of water is absorbed from hypotonic solutions and that this absorption decreases with

increasing concentration. At isotonicity there is very little fluid absorption. This investigator compares the observed Δ of solutions of various concentrations of sodium chloride, after a period of sojourn in the colon, with the theoretical Δ of pure solutions of the same sodium chloride content. It is observed that the osmotic pressure of the solution is always higher than can be accounted for by the sodium chloride content of the solution. The conclusion is therefore drawn that osmotically active substances pass from the organism into the liquid, regardless of the osmotic pressure of the fluid in the colon. Furthermore, Diena states that the osmotic pressure of the intestinal fluid in all concentrations approaches that of the blood.

Hamburger (6) observes, in the peritoneal and pericardial cavities of rabbits, that hypertonic and hypotonic solutions of various salts (including NaCl) come into osmotic equilibrium with the blood, while isotonic solutions show no change in osmotic pressure.

Leathes and Starling, (7), in an investigation into the mechanism of absorption from the pleural cavity, find that absorption of sodium chloride proceeds according to the partial pressure of the salt.

The experimental methods in this investigation are the same as used in previous experiments of this series (8). All of the work is confined to the colon of dogs. Blood chlorides were determined by the method of McLean and Van Slyke. The blood Δ was determined on the whole blood.

EXPERIMENTAL

In table 1 are tabulated several typical protocols which demonstrate that, if a solution of sodium chloride above (exper. 24, etc.) or below (exper. 25) blood level is introduced into the colon, the concentration approaches that of the blood. This is graphically represented in figure 1. In the low concentrations the solvent passes faster than the solute, in concentrations above the blood content, the solute faster than the solvent. With solutions below blood level, the calculated concentrations of the chlorides in the absorbed fluid show increasing values (exper. 25), approaching the concentration of the solution in the intestine as partial pressure equilibrium is established. On the other hand, with solutions of sodium chloride above blood level, decreasing values are obtained (exper. 24) which approach the same level.

Occasionally, as in experiments 26, 29 and 74, the intestinal contents apparently come into equilibrium at a concentration above the con-

TABLE 1

Partial pressure equilibrium between blood and intestinal chlorides

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	NaCl BLOOD	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	Δ BLOOD	CONCENTRATION ABSORBED NaCl	REMARKS
	minutes	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*					
23	60	93	64	0.93	0.85					0.94	Contents returned
	60	62	39	0.85	0.83					0.88	Contents returned
	60	37	18	0.83	0.77					0.89	Contents returned
	60	103	50	0.76	0.73					0.79	Fresh solution
	60	48	17	0.73	0.68					0.75	Contents returned
24	60	82	65	0.85	0.77					1.15	Contents were made up to volume with solutions of equal concentration
	60	90	60	0.77	0.72					0.87	
	60	73	54	0.72	0.68					0.83	
	60	73	40	0.67	0.64					0.71	
	60	81	29	0.64	0.62					0.65	
	60		18	0.63	0.62						
	60	76	21	0.61	0.62	0.59				0.60	
25	60	91	63	0.33	0.41	0.66				0.15	Contents were made up to volume with solutions of equal concentration
	60	88	64	0.41	0.45					0.30	
	60	88	54	0.45	0.49					0.45	
	60	92	53	0.48	0.50					0.45	
	60	91	44	0.50	0.54					0.46	
	60	89	48	0.54	0.57	0.61				0.51	
26	60	92	59	0.63	0.69	0.62				0.52	Contents were made up to volume with solutions of equal concentration
	60	89	57	0.68	0.70					0.64	
	60	94	62	0.69	0.71					0.65	
	60	91	59	0.72	0.72	0.64				0.72	
29	60	100	52	0.64	0.74			0.535		0.53	Contents were made up to volume with solutions of equal concentration
	60	96	50	0.75	0.75	0.66		0.575		0.75	
35	60	86	35	0.67	0.67		0.443	0.500		0.67	
	60	86		0.65	0.67		0.428	0.501	0.573		Fresh solution
36	60	136	111	0.65	0.68		0.445	0.480		0.52	
	60	97	76	0.68	0.70		0.480	0.520		0.61	Contents returned
	60	62	35	0.70	0.68	0.67	0.520	0.510	0.565	0.73	Contents returned
	60	82		0.68	0.67		0.441	0.480			Fresh solution
	60	186	140	0.88	0.84		0.580	0.565		1.00	Fresh solution
	60	126	100	0.84	0.77		0.565	0.550		1.10	Contents returned
	60	86	58	0.77	0.74		0.550	0.550	0.563	0.83	Contents returned

TABLE 1—*Continued*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	NaCl BLOOD	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	Δ BLOOD	CONCENTRATION ABSORBED NaCl	REMARKS
	minutes	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*					
74	60	95	59	0.76	0.75		0.465	0.527		0.78	
	30	40		0.75	0.73		0.527	0.559			Contents returned
	60	95	42	0.51	0.63		0.322	0.446		0.42	Fresh solution
	60	100	47.5	0.66	0.70		0.416	0.476		0.63	Fresh solution
	30	99	67	0.71	0.72		0.434	0.478		0.71	Fresh solution
	30	48	28	0.72	0.72	0.64	0.478	0.506		0.72	Contents returned
Effect of spontaneous increase of blood chlorides upon chloride equilibrium											
30	30	93	63	0.80	0.79			0.554		0.82	Contents made up to volume
	30	90	62	0.78	0.78			0.556		0.78	
	30	96	49	0.60	0.71	0.73		0.552		0.49	Fresh solution High blood Cls due to anuria
33	60	178	128	0.81	0.79		0.545	0.556		0.86	
	60	114	73	0.79	0.76		0.556	0.567		0.84	Contents returned
	60	59	33	0.76	0.76	0.80	0.567	0.567		0.76	High blood Cls due to anuria
Effect of intravenous infusion of NaCl upon chloride equilibrium											
27	60	41	21	0.64	0.70	0.65				0.57	
	60	38	13	0.70	0.69					0.70	Fresh solution
	60	42	11	0.69	0.66					0.70	Fresh solution
	60	38	8	0.65	0.64					0.63	Fresh solution
	60	60	23	0.63	0.66					0.61	Fresh solution
	60	61	27	0.63	0.66	0.65				0.61	Fresh solution
Infused intravenous, 144 cc. 10 per cent NaCl over period 3 hours 40 minutes											
	60	63	32	0.64	0.70					0.58	
	60	64	28	0.68	0.78					0.60	Fresh solution
	60	59	52	0.75	0.88	0.97				—	Fresh solution
Behavior in dead animal											
							Change NaCl −loss +gain				
31	60	81	81	0.75	0.72		−24 mgm.				Solution returned each time
	60	71	69	0.72	0.72		−14				Solution returned each time
	60	59	60	0.72	0.73		+13				Solution returned each time

centration of blood chlorides, or as in experiments 36 and 74, especially when fresh solutions at a concentration close to blood level are introduced, the concentration of the intestinal chlorides may rise above that of the blood. These points will be discussed later in the paper.

The Δ of the solution shows an interesting behavior. In experiment 33 the Δ comes to a standstill at 0.567 with a concentration of chlorides

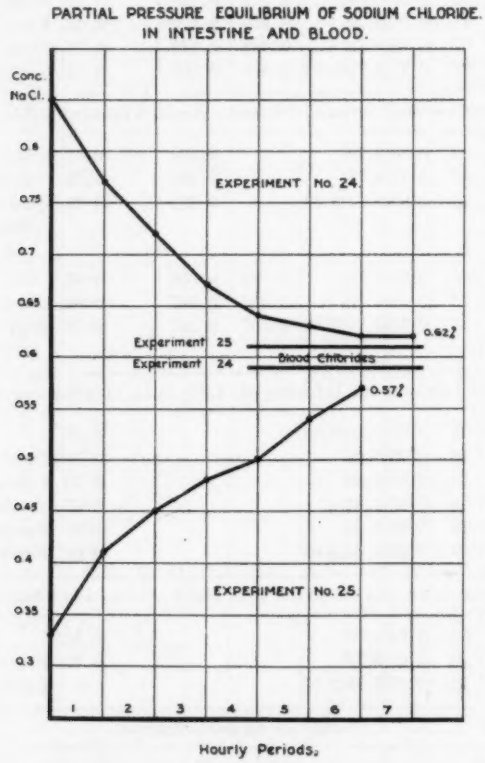

Fig. 1

of 0.76 per cent. On the other hand, in the first periods of experiments 35 and 74, although there is no change of concentration of chlorides, the Δ rises. In the last periods of experiment 36, and the second period of 74, in spite of the fact, that the concentration of chlorides decreases, the Δ rises.

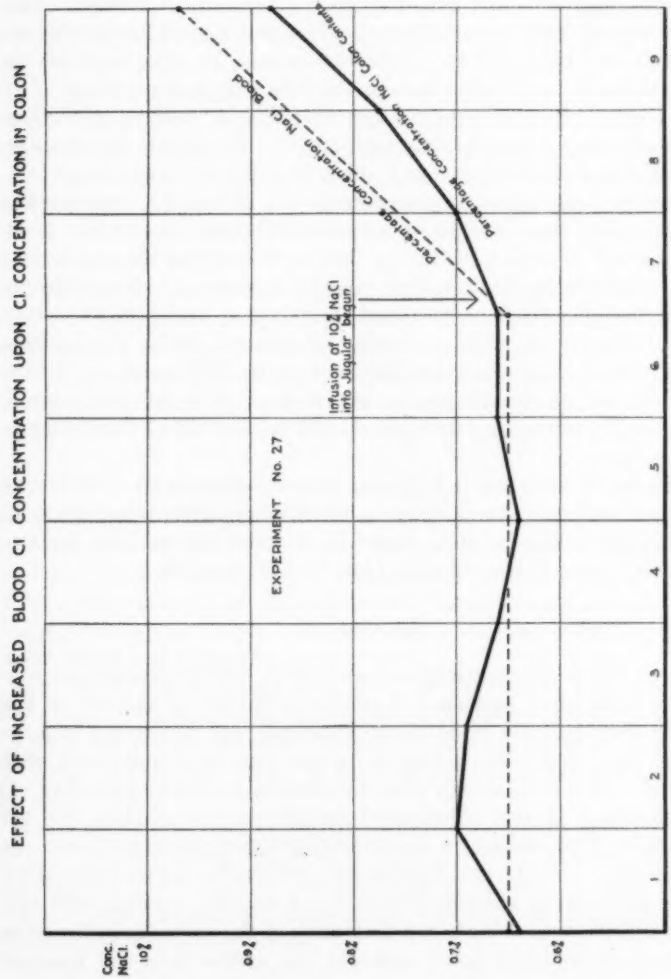
EFFECT OF INCREASED BLOOD Cl CONCENTRATION UPON Cl CONCENTRATION IN COLON
EXPERIMENT No. 27
Infusion of 10% NaCl into Jugular begun
Percentage Concentration NaCl Blood
Percentage Concentration NaCl Colon Contents
Conc. NaCl 10%
0.9%
0.8%
0.7%
0.6%
1
2
3
4
5
6
7
8
9
Hourly Periods

Fig. 2

In the table are given two protocols of experiments in which, early in the period of experimentation, the dogs developed a spontaneous anuria, the result of which was an increase of the blood chlorides. This increase was no doubt in part due to the fact that a considerable amount of chloride was being absorbed from the colon. In experiment 30 the blood rises to 0.73 per cent sodium chloride and in experiment 33 to 0.80 per cent. In each case the concentration of sodium chloride in the gut rises to a comparatively high figure. We merely wish to suggest this as a probable cause and effect in these two experiments for, as we pointed out above in experiments 26, 29 and 74, with normal blood chlorides, there was an apparent equilibrium above blood level.

Experiment 27 shows, definitely, that on raising the blood chlorides by intravenous infusion of sodium chloride a new point of equilibrium between the intestinal contents and the blood is established. A 0.75 per cent solution of sodium chloride introduced during the infusion rises to 0.88 per cent when the blood content is 0.97 per cent. In the normal animal an equilibrium is obtained at 0.66 per cent sodium chloride in the gut with a 0.65 per cent blood content. This is represented in figure 2.

At the end of the table is a typical protocol showing the behavior of solutions of sodium chloride at about blood level, in the colon of a dead animal. The volume of fluid shows little or no change from hour to hour. The same is true of the chloride concentration.

DISCUSSION

The results of the experimental work given above demonstrate conclusively that when solutions of sodium chloride, of greater or less concentration than the blood chloride content, are introduced into the intestine, they come into a partial osmotic pressure equilibrium with the blood. This is in accord with the observations of Gumilewsky (1) and Heidenhain (2) that solutions of sodium chloride of about 0.6 per cent exhibit little change in concentration when introduced into the small intestine. In the two experiments reported by Diena (5) in which 0.92 per cent sodium chloride is introduced into the isolated colon of a dog, there is a decrease in the concentration of the chlorides in the colon content to about blood level, although the author does not mention this point. Our results are in harmony with those of this investigator.

That this equilibrium is related to the blood is further shown by the behavior of gut chlorides, when the blood chlorides spontaneously rise

due to an absorption of chlorides and a diminished excretion through the kidney. That the gut chloride content is dependent upon the concentration of chlorides in the blood, is definitely proven by the establishment of a higher level of equilibrium when the blood chlorides are experimentally increased.

In the dead animals the fluid and chloride absorption show very slight or negative values, hence there is a mere stasis of fluid in the intestine in contrast to an actual interchange in the living animal.

In figure 3 is plotted the observed Δ in the solutions from the colon, superimposed upon the Δ of solutions containing the same concentration of sodium chloride. The latter solutions were made with the same sodium chloride used in the experiments and the Δ determined as with the experimental solutions. The two plots show a wide divergence. The reason for the divergence at the start is the fact that solutions used in our experiments are always first rinsed through the intestine before the sodium chloride content is determined. By this procedure, any dilution from fluid in the colon or any substances which would effect the Δ of the initial solution is taken into account. We can only explain this later divergence, of observed Δ and the Δ determined from actual amount of sodium chloride in the intestinal fluid, on the basis that substances other than chloride, producing osmotic pressure, are present in the gut fluid. Hence, the Δ shows higher values than can be accounted for by the sodium chloride alone. A sysatic determination of these constituents has not been made. Diena (5) reports finding sodium chloride and nitrogenous substances diffusing into glucose solutions in the colon.

Our results are in accord with those of Diena (5), in showing that there is an attempt at a total osmotic equilibrium between the colon contents and the blood. Hamburger (6) showed a similar behavior in the peritoneal and pericardial cavities, and Leathes and Starling (7) in the pleural cavity.

We have tactily assumed in this comparison of intestinal chloride content with equivalent solutions of sodium chloride, that the chlorides estimated in the intestinal contents exist as sodium chloride. However, no other molecular combination, physiologically possible, can adversely affect the conclusion.

We have observed, as mentioned above, first: an apparent equilibrium at a concentration of chlorides above blood level; second: an increase of the Δ of the solutions with little or no change of concentration of the solution; third: a decrease of concentration with no

change in the Δ. These apparent anomalies can be satisfactorily explained by a consideration of the large divergence of the Δ of the two curves in figure 3. It is apparent that as the equilibrium is approached, more and more of substances other than chlorides diffuse into the intestinal contents. Hence, inasmuch as there is an attempt at total os-

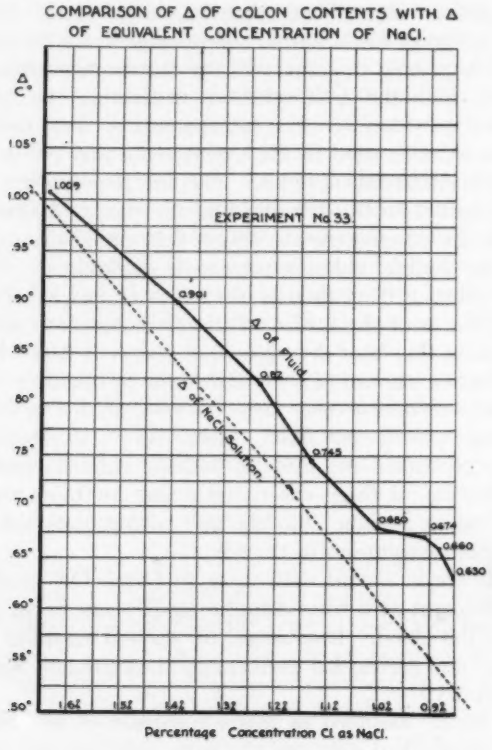

Fig. 3

motic equilibrium as well as partial pressure equilibrium, the concentration of chlorides in the intestine may remain higher than the blood level until replaced by substances but slowly diffusing from the blood. In the cases where the content of the chlorides decreases without a decrease in Δ, other osmotic pressure producing substances have replaced the chlorides.

A protocol given by Diena (5) confirms this point. When he introduces an isotonic solution of sodium chloride into the colon and determines the Δ of the solution recovered, there is little change in the Δ in spite of the fact that there is a considerable decrease in concentration of gut chlorides (from 0.92 to 0.67 per cent). Diena does not comment upon this point.

The observations above may also furnish an analogy and explanation of the findings of Höber that isotonic solutions of sodium chloride occasionally show an increased osmotic pressure during absorption.

CONCLUSIONS

1. Solutions of sodium chloride above or below blood level come into a chloride partial pressure equilibrium with the blood.
2. Increase of blood chlorides affects this equilibrium.
3. There is an attempt at total osmotic pressure equilibrium between the colon contents and the blood.
4. Evidence is at hand which indicates that other blood constituents diffuse into sodium chloride solutions in the colon. This is more apparent as the concentration of the solution approaches the level of the blood chlorides.

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STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

IV. The Colon. The Behavior of Sodium and Magnesium Sulphate Solutions

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In previous papers of this series our investigations have been concerned with the behavior of solutions of sodium chloride of various concentrations. It is evident that the intestinal wall behaves toward solutions of sodium chloride like a permeable membrane. A few of the discrepancies, on the basis of our present knowledge, have been pointed out. A priori, in accordance with the conception of the action of sodium sulphate and magnesium sulphate in producing catharsis, we should expect that the behavior of these salts in the colon would present a contrast to the changes occurring with solutions of sodium chloride. In this paper will be presented the changes occurring in the solvent and solute of solutions of sodium and magnesium sulphate.

There is a paucity of literature on this point, from the quantitative point of view. Some conception of the changes occurring in solutions of sodium sulphate and magnesium sulphate in the small and large intestine may be obtained from the classic work of Mathew Hay. The points bearing upon the subject of this paper will be presented briefly. Hay (1) finds that there is a greater flow of fluid into the large intestine, with a given quantity of sodium sulphate, than into the small intestine, with the same area of intestinal surface exposed to the solution. The author suggests two reasons for this difference. First: the mucous secretion into the large intestine inhibits absorption, and second: from the nature of the mucous membrane of the large intestine absorption proceeds with greater slowness than in the small intestine. If the solution of sodium sulphate is sufficiently strong (20 per cent), Hay finds that the amount of fluid withdrawn from the body may lead to a blood concentration, as indicated by an increased number of red cells.

Furthermore, the work of this author indicates most conclusively that, from the small intestine, at least, considerable amount of sodium sulphate is absorbed into the blood. Nearly half of the amount introduced may disappear in the first hour. This factor is of such importance that, if the dose administered does not exceed what can be absorbed during the rapid stage of absorption, there is a failure of catharsis. The amount of sulphate absorbed is also believed to be responsible for a diuresis following the administration of sodium sulphate.

Finally, this investigator finds an "accumulation" of sulphates in the colon, after administration of sodium sulphate. This he ascribes to a combination of two factors, a slower absorption of the salt from the large intestine, which "depends on a difference in the structure and function of the large intestine as compared with the small intestine," and a passage of salt from the blood into the colon contents.

In regard to magnesium sulphate, Hay (2) believes that the salt is split in the intestine, and sulphate passes more easily than the magnesium. It must be added that Hay is not unaware of the relatively low diffusibility of these salts as a factor in their cathartic action.

Kovesi (3) investigated the absorption of sodium sulphate from the small intestine of rabbits, 120 to 150 cm. from the pylorus, and found considerable disappearance of the sulphate. From hypotonic solutions, fluid is absorbed and there is an increased Δ to the level of the blood. With hypertonic solutions there is an increased volume and a decrease in the Δ. Isotonic solutions show little or no change in volume or Δ. The sulphate is absorbed in rather large amounts, according to the author's figures, the absorption increasing with the concentration of the salt introduced. The length of the experiments varies from 1 to 2 hours.

Gumilewski (4) observed, in the small intestine, that sodium sulphate delayed the fluid absorption and that the sulphate absorption increased with increasing concentration. He concluded that the chemical nature of the salts play a part in their absorption.

Heidenhain (5) performed a few experiments with magnesium sulphate in the small intestine, and finds that water is absorbed more slowly than from a solution of sodium chloride of greater osmotic pressure. He concludes that the sodium sulphate lessens the "physiological activity" of the intestinal cells; hence, the factors involved are controlled more by their osmotic pressures than is the case with solutions of sodium chloride.

Wallace and Cushny (6) compared the fluid absorption from a large number of salt solutions with that of a 1 per cent sodium chloride solution. They find that in the list of the cathartic salts the anion is the determining factor in the absorption. Sulphates, standing between the nitrates and chlorides, retard absorption more than chlorides and are identical with the phosphates. These authors find that solutions of all concentrations tend to become isotonic with the blood. Of the cations, they state that those of the alkaline earths are very slowly taken up by the intestinal epithelium. In this investigation the authors measure only fluid absorption and give no data on the fate of the salt introduced, but assume that compared to sodium chloride relatively little is absorbed.

From the pleural cavity, Leathes and Starling (7) report absorption of sodium and magnesium sulphates.

METHODS

In the experiments to be reported below, the same technique was employed as in our previous investigations. The sodium sulphate used for making solutions was in most cases the anhydrous salt. Glauber's salt was found to give similar results. Sulphates were determined gravimetrically, weighed as barium sulphate. The magnesium was determined by the McCrudden (8) method. At the conclusion of the experiment the colon was washed repeatedly with distilled water, the total volume of the washings was about one liter. In computing the amount of sulphate unaccounted for, the sulphates of the washings and samples are deducted from the total amount introduced initially.

EXPERIMENTAL

The experimental results are appended in tables 1 and 2. Considering first the volume changes of the fluid in the colon, it will be noted that with a hypertonic solution of sodium sulphate (8.15 per cent, exper. 21) there is an increase in volume, which is considerable during the first few hours, finally (after 10 hours) remaining stationary within the range of the error of delivery. In experiment 44, where a strongly hypotonic solution is introduced, there is a very rapid absorption of fluid which continues for six hours.

In experiments 46 and 47, where solutions of 2.01 per cent sodium sulphate are used, there is no considerable volume variation in either case; with the exception of the first period of experiment 46, most of

the changes are not considerably beyond the experimental error of delivery of the fluid.

The volume changes in the magnesium sulphate experiment cannot be discussed in connection with this salt alone, inasmuch as, for other

TABLE 1

The behavior of Na_2SO_4 *in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME — LOSS + GAIN	Na_2SO_4 INTRODUCED	Na_2SO_4 RECOVERED	Na_2SO_4 INTRODUCED	Na_2SO_4 RECOVERED, FINAL SOLUTION, SAMPLES, WASHINGS	DEFICIT Na_2SO_4	Δ BLOOD	Δ CONTENTS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*grams*	*grams*	*grams*		
21	60	25	57	+32	6.64	3.25					
	60	55	72	+17	3.25	2.46					
	60	69	80	+11	2.46	—					
	60	80	87	+ 7	—	2.05					
	60	82	85	+ 3	2.05	—					
	60	85	86	+ 1	—	1.94					
	60	81	75	− 6	1.94	—					
	60	75	77	+ 2	—	1.91					
	60	72	71	− 1	1.91	—					
	60	71	70	− 1	—	1.95	2.445	2.050	0.395		
44	60	250	155	−95	0.50	—				0.582	
	60	155	95	−60							
	60	95	82	−13							
	60	82	48	−34							
	60	48	34	−14							
	60	34	30	− 4		1.65	1.250	1.044	0.206	0.577	0.558
46	60	150	138	−12	2.01	—				0.610	
	60	138	141	+ 3							
	60	141	141	0							
	60	141	139	− 2							
	60	139	140	+ 1							
	60	140	144	+ 4		1.85	3.015	2.778	0.237		0.614
47	60	100	98	− 2	2.01					0.580	
	60	98	99	+ 1							
	60	99	100	+ 1							
	60	100	98	− 2							
	60	98	100	+ 2							
	60	100	100	0		1.56	2.012	1.674	0.338	0.580	0.578

experimental purposes, sodium chloride was present in the initial solutions. However, in the last period we know from analysis that sodium chloride was present in but very small amount (0.06 per cent

or 0.071 gram). Hence, we may say that the volume increases to a constant level, due to the osmotic pressure of the magnesium sulphate.

The changes in concentration of sodium sulphate in experiments 21 and 44 parallel the changes in volume. In the former (hypertonic solution) the concentration decreases rapidly and becomes fixed at about the same time as the volume. In the latter (hypotonic solution) the concentration is increased during the period of the experiment.

In experiments 46 and 47 there is the anomaly of little fluid change, yet in the latter there is an undoubted diminution of the concentration.

With magnesium sulphate, in experiment 68, on introduction of a

TABLE 2

The behavior of $MgSO_4$ *in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME, − LOSS, + GAIN	$MgSO_4$ INTRODUCED	$MgSO_4$ RECOVERED	$MgSO_4$ INTRODUCED	$MgSO_4$ RECOVERED	DEFICIT $MgSO_4$	Mg INTRODUCED	Mg RECOVERED	Δ BLOOD	Δ CONTENTS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*grams*	*grams*	*grams*	*grams*	*grams*		
68	60	100	113	+13	3.56								
	60	113	125	+12									
	60	125	110	−15									
	60	110	118	+ 8									
	60	118	118	0									
	60	118	118	0									
	60	118	118	0		2.80	3.572	3.468	0.104	0.713	0.714	0.583	0.610

hypertonic solution there is a decrease in concentration during the course of the experiment.

Inasmuch as, in this work, our primary interest centered around the differences in the initial and final solutions, the changes in concentration and osmotic pressure of the solutions were not determined from hour to hour.

The final Δ of the solutions is determined in experiments 44, 46, 47 and 68. In every case it closely approximates the blood Δ, although it will be noted in the first three experiments that the concentration of sulphate in the final solution varies. In experiment 46 a chloride estimation in the final solution shows a concentration of 0.01 per cent or a total of 14 mgm. Also in experiment 68 the chloride concentration in the final solution is 0.06 per cent a total of 70 mgm. The final Δ,

therefore, is not, in any considerable part, due to chlorides as we shall show in a following paper.

The amount of sodium sulphate or magnesium sulphate unaccounted for at the end of the experiment is computed. For the sodium sulphate this varies from 200 to 400 mgm. in a period of six to ten hours. In experiment 21, 2.445 grams are introduced with a deficit of 395 mgm. in ten hours.

In experiment 46, the initial solution contained 3.015 grams, and 237 mgm. remained unaccounted for after six hours. In experiment 44, when but 1.25 grams are present, there is a deficit of 206 mgm. in six hours.

In the above calculations the sulphate determined is computed as sodium sulphate for convenience of comparison, but does not imply that all the sulphate was present in the colon as sodium sulphate.

Experiment 68 shows but a slight loss, 104 mgm. of magnesium sulphate (equivalent to 83 mgm. of sulphate). Magnesium was also determined and recovered quantitatively. The grade of sulphate absorption from magnesium sulphate is therefore very low, these figures indicating that it is below the sulphate absorption from solutions of sodium sulphate. None of the magnesium, from this single experiment, appears to be absorbed through the colon.

DISCUSSION

The experiments presented show that hypertonic solutions of sodium or magnesium sulphate gain in volume in the colon of the dog. The concentration decreases and the Δ approaches that of the blood. Hypotonic solutions of sodium sulphate decrease in volume, the concentration rises and the Δ approaches blood level. Solutions of a concentration of sodium sulphate about iso-osmotic with the blood, exhibit little change in volume and approach the Δ of the blood. In this we are in accord with the observations of Kovesi (3), and also those of Wallace and Cushny (6), who showed a similar behavior in the small intestine. The difference of the final concentration of sodium sulphate in the separate experiments is yet to be accounted for. We have shown that chlorides are not present, hence the presence of sodium chloride cannot be the explanation.

The concentration of sodium sulphate equi-osmotic with a 0.95 per cent sodium chloride solution is 1.87 per cent, calculated from the figures of Landolt-Börnstein. For magnesium sulphate it is 2.80 per

cent. It will be noted that the final concentrations, in the solutions in the table above, closely approximate these figures.

Only a very small amount of sulphate is absorbed from sodium sulphate solutions in the colon. The work of Mathew Hay (1), (2) indicates that a large amount of sulphate is absorbed from solutions of sodium sulphate in the intestine, for the most part, while the solution is still in the small intestine. This author believes that there is an accumulation of the salt in the large intestine, due to a slow rate of absorption in this region, and to a passage into the large intestine of sulphate from the blood, since the blood is high in content of this salt from absorption from the small intestine. Our results are in complete harmony with the experimental findings of Hay although, on the basis of our studies, we wish to present a somewhat different interpretation of these results. We would suggest that the accumulation of salt in the colon in Hay's experiments is due entirely to sodium sulphate entering from the small intestine, and that this accumulation is only dependent upon the very slight permeability of the colon to the salt. The supposition of passage of sulphate from the blood into the colon, an unlikely phenomenon, is therefore unnecessary. That the volume of the solution is maintained is also due to this fact and not, as Hay (1), (2) suggested, to the mucous content of the colon fluid, for in our experiment with a clean colon this factor was not observed.

On this basis we wish to suggest, with added proof to that already offered by Hay, that this factor of exceedingly slight disappearance of sodium sulphate points to the colon as playing a very important rôle in catharsis. Providing the cathartic salt reaches the colon in sufficient amount, so that by osmotic interchange between the blood and colon enough fluid is introduced to increase the bulk and fluidity of the colon contents, catharsis will be assured. This might occur independent of the volume of fluid reaching the colon from the small intestine. The determining factor will be the amount of the salt which reaches the large intestine, and inasmuch as little is absorbed, this will be cumulative. Furthermore, once enough of an isotonic or hypertonic solution has reached the large intestine to increase the bulk and fluidity of the colon contents, catharsis is assured, for we have shown that but a small quantity of the salt is absorbed in ten hours.

The very small amount of sulphate which remains unaccounted for in our experiments bears no constant relationship to the amount introduced, in fact the figures are of the same order, independent of time of sojourn or concentration of the initial solution. This leads us to believe

that we are not dealing with an absorption phenomenon. The other possibility which occurs to us is the adsorption of a rather definite amount of sodium sulphate by the intestinal wall, which may be proportional to the final rather than the initial concentration of sodium sulphate. Hofmeister (9) has shown that colloid substances (agar-agar and gelatine) as well as dried tissue, may take up fluid and salt of solutions in which they are soaked. This varies with different salts. At the end of our experiments the intestine was washed with distilled water, yet there is still the possibility that any sulphate so held by the tissue would escape the washing process.

The very low permeability of the large intestine for magnesium is in accord with the findings of Hay (1), (2) on the elimination of magnesium sulphate. Hay suggests that the magnesium remains in the intestine and that sulphate is absorbed and probably excreted as a sodium compound. It also agrees with the findings of Wallace and Cushny (6) who report "very slow" absorption of the cations of the alkaline earths.

Hofmeister (10) suggests that the cathartic action of magnesium sulphate is due to the formation of magnesium carbonate and sodium sulphate in the intestine. Such an assumption is not necessary on the basis of our work.

CONCLUSIONS

1. The colon behaves toward solutions of sodium sulphate essentially like a semi-permeable membrane. Water is absorbed from hypotonic solutions, and the Δ increases to blood level. With hypertonic solutions the volume increases, the concentration decreases and the Δ approaches blood level. Solutions of concentration nearly iso-osmotic with the blood show little change in volume and the Δ closely approximates the Δ of the blood. Hence, there is free passage of water with practically no diffusion of sulphate.

2. The deficit of sodium sulphate is very small and bears no constant relationship to the total amount of sodium sulphate introduced, nor is it dependent upon the length of stay in the colon, and therefore seems to be due to adsorption rather than to a diffusion.

3. Magnesium sulphate shows even less absorption from the colon than sodium sulphate.

4. The failure of absorption of these salts from the colon emphasizes the specific importance of the large intestine in saline catharsis.

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STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

V. The Colon. The Effect of Sodium Sulphate upon the Absorption of Sodium Chloride when the Salts are Introduced Simultaneously into the Intestine

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In the preceding paper of this series it has been shown that the colon acts toward solutions of sodium sulphate and magnesium sulphate essentially like a semi-permeable membrane. To sodium chloride solutions, on the other hand, the colon resembles in many respects a permeable membrane. The cause of this difference in behavior to the same surface is of extreme interest. Is it due to the effect of the salt, per se, upon the cells or walls of the colon whereby the permeability of the colon is altered? This is indeed one possibility which it is our purpose to put to a test in the following experiments.

Starling (1), discussing intestinal absorption, says:

> If, on the other hand, salts, such as sodium sulphate, which pass with extreme difficulty through the cell lining are present, the osmotic pressure, due to the dissolved salts, continues as a force opposing the absorptive activity of the cells.

Heidenhain (2) finds that there is less absorption of water from magnesium sulphate solutions, in the small intestine, than from sodium chloride solutions of equal or even greater osmotic pressure. The author points out that, if water absorption from the intestine were dependent upon osmotic pressure factors, equi-osmotic solutions should have the same water absorption. Hence, Heidenhain ascribes this action of magnesium sulphate to an influence upon the "physiological activity" of the intestinal cells. In order to increase the osmotic pressure of solutions introduced into the small intestine, without changing the amount and concentration of sodium chloride, this investigator adds "an indifferent neutral salt, sodium sulphate." He states

that both the absorption of fluid and of chloride may diminish. The author adds that the action is purely physical and disappears if the intestine is well washed.

Wallace and Cushny (3) discuss the probable cause of the non-absorption of the cathartic salts, and suggest as a possible explanation the precipitation of calcium in the intestinal wall by these salts. They give as analogies the effect of the precipitation of calcium in coagulation of blood and of milk. These investigators state that while sodium sulphate does not, as a general rule, impair the absorption of intestinal loops permanently, they infer that there is an injurious effect.

Hay (4) discusses the effect of solutions of sodium sulphate upon the absorptive power of the intestine. In order to test this point he injects strychnine into sodium sulphate solutions, in ligatured loops of the small intestine of cats. Hay concludes that the intestine retains its powers of absorption even when a 20 per cent solution of a purgative salt has been used, and also that the absorptive power of the intestine is less injured by a weak solution than by a strong solution of the salt. He further believes to have demonstrated that strong solutions of sodium sulphate retard absorption of weak solutions subsequently introduced.

Waymouth-Reid (5) finds that when sodium chloride is added to glucose solution in the small intestine, the uptake of glucose is favored at the same time that water is retarded by the salt. He concludes that this is evidence that "chemical excitation" may play a part in intestinal absorption.

Hamburger (6), in a study of the changes in volume of isolated epithelial cells of the small intestine, subjects them to sodium chloride and magnesium sulphate solutions. Magnesium sulphate in increasing concentration swells the cells. When the cells are merely soaked in magnesium sulphate, and then subjected to increasing concentrations of sodium chloride, Hamburger finds that there is a greater power of diminishing the volume of the cells, than when the cells are merely subjected to sodium chloride alone in increasing concentration. He believes that magnesium sulphate alters the state of permeability of the cells.

METHODS

The experimental procedures and methods of analysis employed in this investigation are similar to those in previous papers of the series. In comparing the total amount of chlorides absorbed when increasing

amounts of sodium sulphate are added to the solution, the concentration of chlorides, volume of solution introduced and length of stay in the intestine, have been kept constant. Hence, the only variables are the sodium sulphate and osmotic pressure of the solution. A low concentration of sodium chloride has been selected for this purpose in order to give as large a range of concentration as possible below the osmotic pressure of the blood, and thus avoid the interfering factor of fluid inflow from the blood. The sodium sulphate was the anhydrous salt.

EXPERIMENTAL

The data in table 1, above, demonstrate that when a solution containing sodium sulphate and sodium chloride is introduced into the colon, the chlorides are absorbed to completion, while the sulphate is

TABLE 1

The effect of Na_2SO_4 *upon diffusion of* NaCl *through colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME: − LOSS, + GAIN	$NaSO_4$ INTRODUCED	$NaSO_4$ RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	Δ CONTENTS	Δ BLOOD	
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*			
34	60	96	94	− 2	2.00		0.92	0.49			
	60	90	77	−13			0.49	0.30			Contents returned
	60	73	61	−12			0.30	0.15			Contents returned
	60	51				1.72	0.15	0.04	0.585	0.590	Contents returned
48	60	80	90	+10	2.00		0.60		0.945	0.596	Contents returned
	60	90	94	+ 4							Contents returned
	60	94	93	− 1							Contents returned
	60	93	87	− 6				0 06			Contents returned
	60	77	74	− 3		1.83	0.06	0.01	0.620	0.590	

responsible for nearly the total osmotic pressure equilibrium between the gut contents and the blood. The initial concentration of sodium sulphate is that of a solution slightly hypertonic with the blood. In both experiments the final concentration of sodium sulphate approaches the concentration of a solution equi-osmotic with the blood. The final Δ of the gut contents also shows little variation from that of the blood. Since examples for analysis were removed at the end of each period we

cannot get a proper picture of the changes in the volume of fluid, but it is evident that they are slight except in the first periods.

Of greater interest is the change in the sodium chloride content. In experiment 34, the initial solution introduced contained 0.92 per cent sodium chloride, there is a rapid and progressive decrease in concentration until the final solution contains but 0.04 per cent. A solution of 0.6 per cent (480 mgm.) was introduced in experiment 48 and the final solution contained but 0.01 per cent (7 mgm.). During the last hour of this experiment the solution was repeatedly removed and returned so that absorption was facilitated by mixing.

TABLE 2

Effect of $MgSO_4$ *upon diffusion of* NaCl *through colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME	$MgSO_4$ INTRODUCED	$MgSO_4$ RECOVERED	NaCl INTRODUCED	NaCl RECOVERED	Δ CONTENT	Δ BLOOD	REMARKS
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*			
50	60	90	81	− 9	1.00		0.81	0.67			Solution of $MgSO_4$ undergoing concentration with disappearance of NaCl
	60	71	58	−13			0.67	0.54			
	60	48	29	−19		1.57	0.54	0.39			
68	120	85	125	+40	3.56		0.82				Solution of $MgSO_4$ undergoing dilution with disappearance of NaCl
	120	125	118	− 7							
	180	118	118	0		2.80		0.06	0.610	0.583	

Table 2 shows two experiments which demonstrate the behavior of sodium chloride in the presence of magnesium sulphate in the large intestine. These data present precisely the same features as are shown in table 1 with sodium sulphate. With a concentration of magnesium sulphate hypertonic with the blood (exper. 68,) the chlorides are rapidly absorbed and the amount present rapidly approaches a zero value (0.06 per cent or 71 mgm.). The concentration of magnesium sulphate is nearly that of a solution equi-osmotic with the blood. The Δ of the final solution closely approximates that of the blood.

With a concentration of magnesium sulphate hypotonic with the blood (exper. 50,) both fluid and chlorides are rapidly absorbed. There is every indication that if the experiment were continued for a suffi-

cient period of time, the chlorides would diminish to a very low figure, and the magnesium sulphate reach a concentration of a solution equiosmotic with the blood.

It has been shown in previous work that chlorides pass into distilled water in the large intestine (7). In table 3 have been compiled a few typical protocols which show the degree to which this phenomenon occurs when the fluid in the colon is sodium sulphate. These data demonstrate that the total amount of chlorides passing into dilute solutions of sodium sulphate is less than that passing into water (exper. 40) and that the percentage concentration is also lower. Furthermore, it will be noted that the order of magnitude of chloride concentration and total amount is less in solutions of sodium sulphate as the concentration of the latter increases (exper. 41, 33 and 34.)

TABLE 3

The passage of chlorides into solutions of sodium sulphate in colon

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	Na_2SO_4 INTRODUCED	NaCl RECOVERED	AMOUNT NaCl RECOVERED
	min.	*cc.*	*cc.*	*per cent*	*per cent*	*grams*
40	30	100	90	0	0.09	0.081
	30	100	88	0	0.08	0.070
	30	100	87	0.15	0.05	0.043
	30	100	87	0.15	0.03	0.026
	30	100	83	0.15	0.04	0.033
	30	95	82	0.30	0.03	0.025
41	30	140	81	0.50	0.01	0.011
33	120	195	126	1.00	0.02	0.24
34	45	100	95	2.00	0.02	0.16

Up to this point it has been shown that sodium chloride in the presence of either sodium or magnesium sulphate in the large intestine is absorbed and after a time approaches a zero value. Further experiments show that sodium sulphate influences the speed of absorption of sodium chloride. The results are given in table 4.

In the experiment presented (79), increasing concentrations (0.1 to 2.0 per cent) of sodium sulphate, containing a constant concentration of sodium chloride, are introduced into the intestine. Since the volume of the solutions introduced is constant, the total amount of sodium chloride in each solution is, therefore, the same. The time which the solution is allowed to remain in the intestine is also the same in all periods. The range of osmotic pressure in the sodium sulphate solu-

tions is from a strongly hypotonic to hypertonic, as compared to the blood. Experimental periods (sodium sulphate) are always alternated with control periods (sodium chloride alone). After each period in which sodium sulphate has been used, the colon is washed with saline

TABLE 4

Effect of sodium sulphate upon the speed of diffusion of NaCl *in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	VOLUME CHANGE; + GAIN, − LOSS	Na_2SO_4 INTRODUCED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl; + GAIN, − LOSS	CHANGE NaCl; − LOSS	Δ SOLUTION INTRODUCED	Δ CONTENTS RECOVERED	CONC. NaCl SOLUTION ABSORBED
Experiment 79	*mins.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*			*per cent*
Period 1	30	100	76	−24	0	0.3	0.36	+0.06	−26	0.210	0.275	0.11
	30	100	75	−25	0	0.3	0.35	+0.05	−37	0.205	0.265	0.15
	30	100	75.5	−24.5	0	0.3	0.35	+0.06	−36	0.200	0.256	0.15
	30	100	71.5	−28.5	0.10	0.3	0.33	+0.03	−64	0.228	0.297	0.23
Period 2	The gut washed with 300 cc. of 0.3 per cent NaCl solution											
	30	100	71.5	−28.5	0	0.3	0.34	+0.04	−57	0.206	0.252	0.20
	30	100	76	−24	0.50	0.29	0.28	−0.01	−77	0.353	0.404	0.32
Period 3	Gut washed with 400 cc. of 0.3 per cent NaCl solution											
	30	100	70	−30	0	0.3	0.35	+0.05	−55	0.210	0.259	0.18
	30	100	91	− 9	1.0	0.3	0.24	−0.06	−82	0.498	0.513	0.91
Period 4	Gut washed with 400 cc. 0.3 per cent NaCl solution											
	30	100	72	−28	0	0.3	0.35	+0.05	−48	0.202	0.256	0.17
	30	100	100	0	1.5	0.3	0.22	−0.08	−80	0.646	0.611	
Period 5	Gut washed with 400 cc. of 0.3 per cent NaCl solution											
	30	100	71	−29	0	0.3	0.36	+0.06	−44	0.195	0.260	0.15
	30	100	107	+ 7	2.0	0.29	0.21	−0.08	−65	0.775	0.709	
Period 6	Gut washed with 400 cc. of 0.3 per cent NaCl solution											
	30	100	72.5	−27.5	0	0.30	0.36	+0.06	−39	0.195	0.258	0.14
	30	100	75	−25	0	0.30	0.33	+0.03	−52	0.198	0.236	0.21

(0.3 per cent), in order to remove as much as possible of the sodium sulphate.

It will be noted that, with increasing concentration of sodium sulphate, the total amount of chlorides absorbed, in every case, exceeds that of the corresponding control period. This is shown graphically

in figure 1. This is true regardless of whether the experimental solutions are hypotonic (periods 1, 2 and 3) or hypertonic (periods 4 and 5.) The volume of fluid absorbed, on the other hand, is progressively decreased, so that the calculated concentration of the chloride solution absorbed shows increasing values in the experimental periods. It should be observed that whereas in the control periods the final concentration of sodium chloride is always above that of the initial solution, the reverse is the case when sodium sulphate is added in the

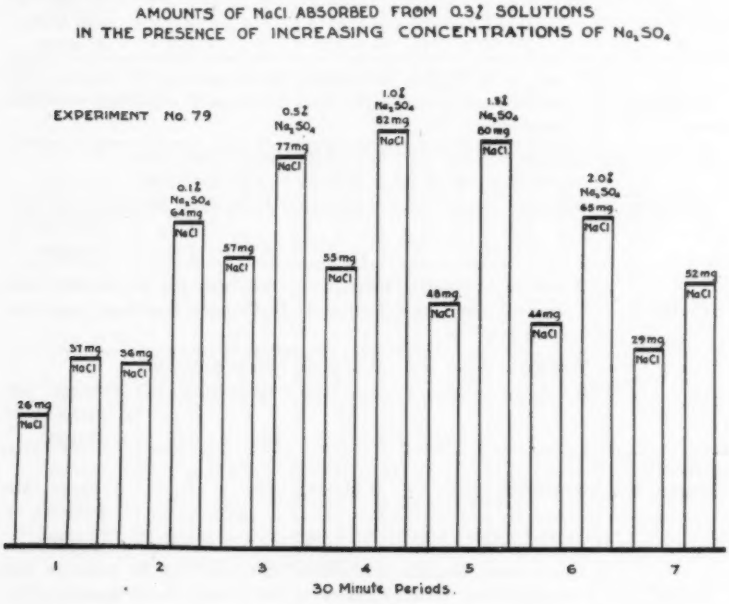

Fig. 1

experimental periods. As will be seen from the table, this diminution in the concentration of chlorides increases as the concentration of sodium sulphate is raised in the experimental solution.

Another point is evident when one compares the amount of chlorides absorbed in the different control periods (table 4). The control of period 2, which follows the first period in which sodium sulphate is used, shows an undoubted increase of chloride absorption over the preceding control periods. This higher level of chloride absorption

TABLE 5

Effect of sodium sulphate upon colon wall

EXPERIMENT NUMBER	TIME	INTRODUCED VOLUME	RECOVERED VOLUME	CHANGE VOLUME; − LOSS, + GAIN	Na_2SO_4 INTRODUCED	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl IN CONTENTS; − LOSS	Δ FLUID INTRODUCED	Δ FLUID RECOVERED	REMARKS
	mins.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*mgm.*			
Experiment 80											
Period 1..	30	100	77	−23	0	0.31	0.33	−56	0.216	0.250	
	30	100	78	−22	0	0.31	0.33	−53	0.214	0.254	
	30	100	75.5	−24.5	0	0.31	0.33	−61	0.212	0.247	Average absorption = 57 mgm.
Period 2..	2 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 3..	30	100	159	−41	0	0.31	0.38	−86	0.208	0.290	
	30	100	67.5	−32.5	0	0.31	0.35	−74	0.208	0.268	
	30	100	67	−33	0	0.31	0.35	−75	0.206	0.265	Average absorption = 78 mgm.
Period 4..	2 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 5..	30	100	67	−33	0	0.31	0.35	−75	0.213	0.270	
	30	100	65	−35	0	0.31	0.35	−82	0 215	0.265	Average absorption = 78 mgm.
Experiment 81											
Period 1....	30	100	62	−38	0	0.31	0.36	−87			Average absorption = 87 mgm.
Period 2..	1 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 3..	30	100	56	−44	0	0.30	0.37	−93			
	30	100	62	−38	0	0.31	0.37	−81			Average absorption = 87 mgm.
Period 4....	30	100	79	−21	1.0	0.31	0.25	−112			
Period 5....	30	100	62	−38	0	0.31	0.37	−81			
Period 6....	2 per cent Na_2SO_4 introduced into gut for 30 minutes and washed out with 0.3 per cent NaCl until washings were free from SO_4										
Period 7....	30	100	63	−37	0	0.31	0.38	−71			

persists in the remaining control periods of the experiment. Table 5 (above) gives two typical experiments which demonstrate that the effect just described is a constant one after sodium sulphate has been in the colon.

TABLE 6

Effect of sodium sulphate contrasted with mechanical washing and various concentrations of NaCl *and distilled water upon the colon wall*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME: −LOSS +GAIN	NaCl INTRODUCED	NaCl RECOVERED	CHANGE NaCl: −LOSS	REMARKS
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*mgm.*	
Experiment 82								
Period 1...	30	100	78	−22	0.31	0.34	−45	
Period 2...	Washed intestine with 950 cc. of 0.31 per cent NaCl solution							
Period 3...	30	100	76	−24	0.31	0.34	−52	
Period 4...	Washed intestine with 500 cc. of 0.31 per cent NaCl solution							
Period 5...	30	100	75.5	−24.5	0.31	0.34	−53	
Period 6...	Allowed 1 per cent NaCl to remain in gut for 30 minutes							
Period 7...	30	100	71.5	−28.5	0.31	0.35	−60	
Period 8...	30	100	91	− 9	0.31	0.26	−83	Solution contained 1 per cent Na_2SO_4. Note increased NaCl loss
Experiment 83								
Period 1...	30	100	80	−20	0.31	0.34	−38	
Period 2...	Washed intestine with 400 cc. of 0.31 per cent NaCl solution							
Period 3...	30	100	77.5	−22.5	0.30	0.33	−44	
Period 4.	Washed intestine with distilled water and allowed it to remain in gut for 30 minutes							
Period 5...	Then washed intestine with 200 cc. of 0.31 per cent NaCl							
Period 6...	30	100	80	−20	0.30	0.32	−44	
Period 7.	Washed intestine with 0.6 per cent NaCl solution and allowed it to remain in gut for 30 minutes. Then washed intestine with 200 cc. of 0.31 per cent NaCl							
Period 8...	30	100	74	−26	0.30	0.33	−56	
Period 9	Allowed 1.98 per cent NaCl to remain in gut 30 minutes. Then washed intestine with 250 cc. of 0.31 per cent NaCl solution							
Period 10..	30	100	73	−27	0.30	0.35	−44	

There is little doubt that in period 3, experiment 80, after a 2.0 per cent sodium sulphate solution is allowed to remain for thirty minutes in the intestine, the chloride absorption from solutions of sodium chloride is of a higher grade than in period 1. However, a repetition of this procedure does not increase this latter grade of chloride absorption, as is indicated in periods 4 and 5. This point is further substantiated in experiment 81. Period 1 represents a period following the presence of sodium sulphate in the intestine for other experimental purposes. It can be seen that the amount of chloride absorbed cannot be increased by subsequent treatment of the colon with sodium sulphate. However, the same percentage concentration of sodium sulphate, introduced simultaneously with the sodium chloride, increases the amount of absorbed chlorides greatly in period 4.

It seemed desirable to test whether the effect just described may be due to the mechanical process of repeatedly washing the intestine with salt solution, giving a cleaner absorptive surface. In addition, it is of interest to know whether the effect, whatever its cause, is peculiar to sodium sulphate, or whether treatment of the intestine with other solutions than those employed would produce the same result. The results are uniformly negative and are compiled in table 6.

In experiment 82 mechanical washing shows no effect upon the grade of chloride absorption. The amounts of sodium chloride absorbed do not show any marked variation from period to period. In period 8, however, the addition of sodium sulphate gives the characteristic increase of chloride absorption.

In experiment 83 it is shown that neither distilled water, 0.6 per cent, nor 1.98 per cent sodium chloride solution have an effect upon the amount of chlorides subsequently absorbed from solutions of 0.3 per cent of this salt.

These features are discussed below.

DISCUSSION

The data presented above show conclusively that when sodium chloride and either sodium sulphate or magnesium sulphate are present in the colon, the amount of chlorides decreases. The percentage concentration of these sodium chloride solutions progressively decreases, which is a reversal of the normal behavior of solutions of sodium chloride alone. The concentration of sodium or magnesium sulphate approaches that of a solution equi-osmotic with the blood.

The Δ of the solution also approximates that of the blood, thus, the sodium or magnesium sulphate makes up, as far as we may judge at present, the total osmotic pressure equilibrium between the colon contents and the blood.

It has been further shown that smaller quantities of sodium chloride pass into weak solutions of sodium sulphate than into distilled water in the colon. This effect is more exaggerated with increasing concentrations of sodium sulphate. The presence of sodium sulphate in sodium chloride solutions increases the speed of absorption of chlorides. In the course of the experiments certain observations were made which may throw some light upon the cause of this speeding effect. It is noticed that after sulphates have been present in the intestine, the subsequent control periods, with solutions of pure sodium chloride, show an increase in the chloride absorption. This increased chloride absorption rises to a maximum usually after the first introduction of the sodium sulphate solution, and is maintained at almost the same level in all of the following control periods. It is difficult to ascribe this effect to only sulphates retained in the intestine, inasmuch as it is constant, regardless of the concentration of sulphate introduced in the experimental periods. Another factor which we have thought might in part account for this "lag" is the mechanical effect of washing the intestine, but our experiments on this point give negative results.

The possibility arises that the speeding effect is due to an action of the sodium sulphate upon the permeability of the intestinal epithelium. We have tried in other ways to influence the permeability of the membrane, by introduction of hypertonic solutions of sodium chloride and by distilled water, with negative results. Hence, at this stage of our investigations this problem is unsettled.

We wish to suggest, as another possible explanation, that the total osmotic pressure of the solution is an important factor in determining the rate and direction of the passage of diffusible substances through the intestine, whether or not this osmotic pressure is due to diffusible or non-diffusible substances.

The inability of distilled water and high concentrations of sodium chloride (1.98 per cent) to affect the subsequent absorptive capacity of the intestinal wall proves that the intestinal epithelium is not injured. This is of interest in connection with the observations of Waymouth-Reid (8) that, in portions of the small intestine, the epithelium is very susceptible to strength of solution employed and especially to distilled water. He reports there mere washing of a loop of the small intestine

with distilled water shows less absorption from the animal's own serum than a control washed with 0.9 per cent sodium chloride. These observations are not in accord with our experiments.

Within the range of concentration of sodium sulphate, used in our experiments, there is absolutely no evidence that the presence of sodium sulphate injures the intestinal epithelium as suggested by Hay (4) and Wallace and Cushny (3), and thereby impedes absorption of sodium chloride from solutions subsequently introduced.

Our results are also contrary to those of Heidenhain (2), using the small intestine, that there is a retardation of salt absorption when sodium sulphate is added to sodium chloride. In regard to water absorption we are in agreement.

The cause of the failure of passage of sodium sulphate through the colon wall is of utmost interest.

Hamburger (6), from his experiments upon isolated epithelial cells, believes that magnesium sulphate alters the state of permeability of the cells. From our experiments we must conclude that, if this be the case, the effect must be an increased permeability, at least for sodium chloride.

The suggestion of Wallace and Cushny (3) that the probable explanation of the cause of the low absorption of the cathartic salts is the precipitation of calcium in the intestinal wall, is of interest in this connection. If these authors imply that this is the factor influencing the permeability of the epithelium, we cannot agree. For, if this be true, the absorption of sodium chloride as well as sodium sulphate should be impeded. Our experiments show that not only is the absorptive capacity of the intestinal wall for sodium chloride not decreased but, on the contrary, is increased under these conditions.

The effect of sodium sulphate upon sodium chloride is not without analogy in the body. Hamburger (9) finds that on injection of a hypertonic solution of sodium sulphate into the blood of dogs and horses, there is a reduction of the sodium chloride, carbonate and protein content of the serum, although the osmotic pressure of the blood returns to normal very quickly. Hypotonic solutions have the same effect. He believes that this is due to the slowness with which sodium sulphate leaves the blood.

Cushny (10) finds in rabbits that when sodium sulphate and sodium chloride are injected together into the blood stream, the chlorides of the urine rise rapidly not only in absolute amount but also in percentage, followed by a rapid fall. The sulphate excretion, on the other

hand, falls more slowly and is present in the urine after the chlorides have disappeared. Sulphates injected alone also give a large excretion of chlorides. The author's explanation of this phenomenon is the difference in the reabsorption of sodium chloride and sodium sulphate in the kidney. This investigator says that the epithelium of the renal tubules resembles that of the intestine histologically and physiologically in its reaction to chlorides, phosphates and sulphates.

Magnus (11), in a study of the diuresis produced by various salts in dogs and rabbits finds that, when sodium sulphate is injected into the blood, chlorides sink as the sulphate content rises in the blood. He believes that chlorides go into the tissues. This investigator does not observe the increased chlorides in the urine.

SUMMARY

1. When sodium sulphate or magnesium sulphate is added to solutions of sodium chloride and introduced into the colon, the concentration of chlorides diminishes rapidly and the amount present approaches a zero point.

2. The concentration of sodium or magnesium sulphate approaches a value of a concentration equi-osmotic with the blood. The final Δ of the solution approximates that of the blood.

3. Less chlorides diffuse into weak solutions of sodium sulphate than into distilled water and this amount decreases with increasing concentration of sodium sulphate.

4. Sodium sulphate speeds the rate of absorption of sodium chloride through the colon wall when the two are simultaneously introduced. This increases with increasing concentration of the sulphate. It occurs in hypotonic as well as in hypertonic solutions.

5. After sodium sulphate has been present in the intestine the subsequent absorption of sodium chloride and water to some extent increases. It is never decreased.

6. Other factors such as mechanical washing, introduction of various strengths of sodium chloride, and notably distilled water, do not affect the subsequent absorption of sodium chloride or fluid in the colon.

7. Analogies of the effect of sodium sulphate upon chlorides, in other parts of the body, are presented from the literature.

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STUDIES IN THE MECHANISM OF ABSORPTION FROM THE INTESTINE

VI. The Colon. The Influence of Calcium Salts upon the Absorption of Sodium Chloride in the Intestine

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It has been demonstrated that sodium sulphate has a speeding effect upon the diffusion of sodium chloride through the colon wall. One possible explanation of this phenomenon is an effect of the sodium sulphate upon the permeability of the intestinal wall. In view of the well-known antagonistic action of calcium upon sodium chloride, supposedly related to permeability changes, we have studied the effects of calcium salts upon the passage of chlorides out of the colon.

The researches of Loeb (1) lead him to believe that the antagonistic effects described by him are dependent upon the salts concerned preventing each other from entering the cells or to a difference in the rate of diffusion of the salt mixture through the membrane, and not an effect upon the protoplasm.

Osterhout (2) is of the same opinion as regards the hindering effects of the salts on each other, but finds that even balanced solutions may enter the cell, if but slowly. He believes that the antagonistic substances affect certain life processes which control permeability. The preservation of normal permeability according to the investigator is the result rather than a cause of antagonism. This author favors the view of a change in properties of the membrane.

Lillie (3) also believes that the "antitoxic" action of calcium salts upon sodium salts is due to a prevention or retardation of the increase in permeability produced by the latter salts alone.

Mathews (4) states that calcium may increase the permeability of the membrane of the fundulus egg for some ions and reduce it for others.

Clowes (5) reports that salts like $CaCl_2$ diminish the permeability of protoplasmic membranes for water, while salts like NaOH and NaCl increase the permeability of the membrane. Although antagonism is due to a balance between all cations and anions involved, it happens that $CaCl_2$ has a dominant cation while NaCl has a dominant anion.

The inhibitory action of calcium on body phenomena has been demonstrated. MacCallum (6) observed that $CaCl_2$ may exert an inhibitory effect upon the diuretic action of certain hemolytic substances and in addition inhibit the hemolysis. Davis (7) has found that $CaCl_2$ intravenously causes urinary secretion to diminish and will antagonize the diuretic action of sodium chloride.

The following data are presented as a preliminary report.

METHODS

The experimental methods were the same as described in the first paper of this series (8). The calcium lactate $Ca(C_3H_5O_3)_2.5\ H_2O$ was accurately weighed. The composition of all the calcium lactate solutions is expressed as grams of calcium lactate per 100 cc. of solution. The calcium chloride was the anhydrous salt, and the concentrations are similarly expressed.

EXPERIMENTAL

The data, compiled in the protocols of table 1 demonstrate the accelerating effect followed by the inhibitory action of increasing concentration of calcium lactate upon the passage of chlorides of sodium chloride from the colon.

In experiments 2 and 3, with a level of sodium chloride of 0.6 per cent, it will be noted that with increasing concentrations of calcium lactate, a point is reached where the total amount of chlorides absorbed is diminished. This is shown in experiment 2, by a slight diminution of chloride absorption in period 5. A more marked effect is shown in period 6, with a higher concentration of calcium lactate. The solutions of period 5 are isotonic and those of period 6 hypertonic with the blood. In experiment 3, the first slight inhibitory action is in period 6. The next two periods, 7 and 8, in which the calcium lactate concentration is progressively increased, show a marked decrease in the chloride absorption. Period 10, which corresponds in concentration of calcium lactate to period 6, exhibits a decrease from the preceding control period (9). All of these solutions are hypertonic with the blood.

TABLE 1

The effect of Ca *upon water and chloride absorption in the colon*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME FLUID, +GAIN −LOSS	NaCl INTRODUCED	CA LACTATE INTRODUCED	NaCl RECOVERED	CHANGE NaCl, +GAIN −LOSS	CHANGE NaCl −LOSS	△ FLUID INTRODUCED	△ FLUID RECOVERED	RATIO Ca: Cl
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*			
Experiment 2												
Period 1...	30	85	60	−25	0.62	0	0.64	+0.02	−143	0.390	0.414	
Period 2...	30	85	60	−25	0.63	0.1	0.63	0	−158	0.423	0.439	1: 21
Period 3...	30	85	65	−22	0.63	0.3	0.60	−0.03	−146	0.466	0.484	1: 7.0
Period 4...	30	85	69	−16	0.63	0.6	0.56	−0.07	−149	0.527	0.530	1: 3.5
Period 5...	30	85	74	−11	0.62	0.9	0.55	−0.07	−120	0.586	0.586	1: 2.6
Period 6...	30	85	81.5	− 3.5	0.61	1.38	0.57	−0.04	− 54	0.674	0.664	1: 1.5
Experiment 3												
Period 1...	30	85	62.5	−22.5	0.63	0	0.69	+0.06	−104			
Period 2...	30	85	69	−16	0.63	0.42	0.62	−0.01	−108			1: 4.9
Period 3...	30	85	70	−15	0.63	0.63	0.58	−0.05	−130			1: 3.3
Period 4...	30	85	71	−14	0.63	0.85	0.57	−0.06	−131			1: 2.4
Period 5...	30	85	76	− 9	0.63	1.06	0 54	−0.09	−125			1: 1.9
Period 6...	30	85	80.5	− 4.5	0.62	1.42	0.51	−0.11	−117			1: 1.4
Period 7...	30	85	89	+ 4	0.62	2.65	0.49	−0.13	− 91	0.802	0.708	1: 0.8
Period 8...	30	85	91	+ 6	0.62	3.19	0.48	−0.14	− 90	0.946	0.851	1: 0.6
Period 9...	30	85	55.5	−29.5	0.63	0	0.70	+0.07	−147	0.421	0.481	
Period 10..	30	85	78.0	− 7	0.63	1.49	0.53	−0.10	−122	0.630	0.666	1: 1.4
Experiment 4												
Period 1	30	85	64	−21	0.35	0	0.41	+0.06	− 35	0.245	0.300	
	30	85	60	−25	0.35	0	0.41	+0.06	− 52	0.239	0.292	
	30	85	49	−36	0.34	0.35	0.36	+0.02	−113	0.306	0.363	1: 3.2
Period 2	30	85	52.5	−32.5	0.34	0	0.40	+0.06	− 79	0.236	0.278	
	30	85	72	−13	0.34	1.06	0.31	−0.03	− 66	0.436	0.452	1: 1
Period 3	30	85	44	−41	0.34	0	0.40	+0.06	−113	0.238	0.286	
	30	85	75	−10	0.34	1.59	0.30	−0.04	− 64	0.511	0.526	1: 0.7
	Washed intestine with 0.34 per cent NaCl solution to remove traces Ca lactate											
Period 4	30	85	40	−45	0.34	0	0.42	+0.08	−121	0.240	0.306	
	30	85	82	− 3	0.34	2.12	0.30	−0.04	− 43	0.626	0.597	1: 0.5
Period 5	30	85	46	−39	0.34	0	0.41	+0.07	−100	0.232	0.289	
	30	85	84	− 1	0.34	2.50	0.30	−0.04	− 37	0.658	0.655	1: 0.45
Period 6	30	85	52	−33	0.34	0	0.41	+0.07	− 76	0.228	0.293	
	30	85	87	+ 2	0.34	3.00	0.30	−0.04	− 28	0.764	0.730	1: 0.37
Period 7	30	85	58	−27	0.34	0	0.41	+0.07	− 51	0.239	0.273	
	30	85	61	−24	0.34	0	0.39	+0.05	− 51	0.228	0.267	

TABLE 1—*Continued*

EXPERIMENT NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME FLUID +GAIN −LOSS	NaCl INTRODUCED	Ca LACTATE INTRODUCED	NaCl RECOVERED	CHANGE NaCl +GAIN −LOSS	CHANGE NaCl −LOSS	△ FLUID INTRODUCED	△ FLUID RECOVERED	RATIO Ca:Cl
Experiment 5	*min.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*			
Period 1	30	100	68	−32	0.31	0	0.35	+0.04	− 72	0.204	0.269	
	30	96	60	−36	0.31	0	0.35	+0.04	− 88	0.199	0.240	
	30	100	59	−41	0.31	0	0.35	+0.04	−104	0.206	0.242	
	30	100	65	−35	0.31	0	0.35	+0.04	− 83	0.209	0.257	
	30	92	48	−44	0.31	0.1	0.33	+0.02	−127	0.223	0.261	1: 10
Period 2	Washed intestine with 100 cc. 0.3 per cent NaCl solution											
	30	100	61	−39	0.31	0	0.34	+0.03	−103	0.204	0.236	
	30	100	58	−42	0.31	0.3	0.32	+0.01	−124	0.271	0.320	1: 3.5
Period 3	Washed intestine with 150 cc. 0.31 per cent NaCl solution											
	30	100	60	−40	0.30	0	0.36	+0.06	− 84	0.213	0.261	
	30	100	72	−28	0.30	0.6	0.30	0	− 84	0.332	0.372	1: 1.7
Period 4	Washed intestine with 150 cc. 0.31 per cent NaCl solution											
	30	100	60	−40	0.30	0	0.36	+0.06	− 84	0.209	0.257	
	30	100	77	−23	0.30	0.9	0.29	−0.01	− 77	0.376	0.406	1: 1.1
Period 5	Washed intestine with 200 cc. 0.31 per cent NaCl solution											
	30	100	54	−43	0.31	0	0.36	+0.05	−116	0.202	0.264	
	30	100	82	−18	0.30	1.2	0.27	−0.03	− 79	0.420	0.443	1: 0.8
Period 6	Washed intestine with 250 cc. 0.31 per cent NaCl solution											
	30	100	55	−45	0.31	0	0.35	+0.04	−118	0.209	0.251	
	30	100	99	− 1	0.30	2.4	0.25	−0.05	− 53	0.621	0.581	1: 0.4
Period 7	Washed intestine with 300 cc. 0.31 per cent NaCl solution											
	30	100	58	−42	0.30	0	0.35	+0.05	− 97	0.209	0.259	
	Washed intestine with 350 cc. 0.31 per cent NaCl solution											
	30	100	57	−43	0.31	0	0.35	+0.04	−111	0.210	0.279	

In order to afford a greater range of concentrations below isotonicity, the concentration of sodium chloride in the next two experiments is decreased. In addition, each alternate period is made a control, keeping constant the concentration and amount of sodium chloride.

Experiments 4 and 5 give some new features and bring out more clearly what has been indicated in the preceding experiments. In both experiments two effects are observed when calcium lactate, in increasing concentrations, is added to sodium chloride. First: at low

concentrations of calcium lactate, the amount of chlorides absorbed is increased. This is evident in period 1 of experiment 4 and periods 1 and 2 of experiment 5. Second: at higher concentrations of calcium lactate there is an opposite effect, namely, a decrease in the amount of chlorides absorbed. In experiment 4, all of the experimental periods, after 1, show this inhibitory effect. It is also demonstrated, in experi-

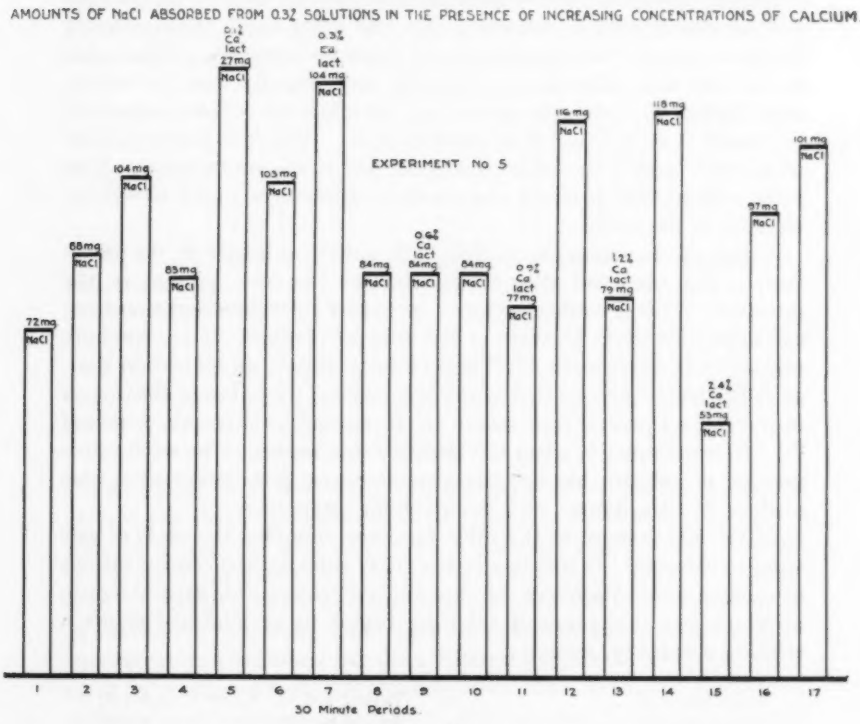
AMOUNTS OF NaCl ABSORBED FROM 0.3% SOLUTIONS IN THE PRESENCE OF INCREASING CONCENTRATIONS OF CALCIUM.
EXPERIMENT No. 5
72mg NaCl
68mg NaCl
104mg NaCl
85mg NaCl
0.1% Ca lact 27mg NaCl
103mg NaCl
0.3% Ca. lact. 104mg NaCl
84mg NaCl
0.6% Ca lact 84mg NaCl
84mg NaCl
0.9% Ca lact 77mg NaCl
116mg NaCl
1.2% Ca lact 79mg NaCl
118mg NaCl
2.4% Ca lact 53mg NaCl
97mg NaCl
101mg NaCl
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
30 Minute Periods.

Fig. 1

ment 5, in the experimental periods following no. 3. An intermediate effect is seen in period 3 of experiment 5. With the concentration of calcium lactate used in this solution, the amount of chlorides absorbed does not differ from that of the control period where sodium chloride alone is subjected to absorption. All of these changes may occur in solutions of osmotic pressure below that of the blood. A graphic representation of a typical experiment is given in figure 1.

The fluid absorption may, at first, show a slight increase over the control periods. This is indicated in period 1 of experiment 4 and in periods 1 and 2 of experiment 5. This occurs coincidently with the increased chloride absorption. Thereafter there is a progressively decreasing fluid absorption, from the experimental solutions, with increasing concentrations of calcium lactate.

During absorption the concentration of sodium chloride in the control periods is always increased. On the other hand, when calcium lactate is present the concentration of chlorides decreases. This is due to the fact that chlorides are absorbed more rapidly than the water, even though the total absorption may be inhibited. This is observed in periods 2, 3, 4, 5 and 6 of experiment 5. With low concentrations of calcium lactate the effect just described is less pronounced. This same phenomenon is noted when sodium sulphate is added to sodium chloride in the colon.

A lag effect, analogous to that with sodium sulphate in the intestine, is also observed after calcium lactate has been present in the intestine. This is evidenced by an increased chloride absorption from solutions of sodium chloride in the control periods. It is especially noticeable in experiment 4. There is, furthermore, an indication that, after the high concentration of calcium lactate, the chloride absorption in the control periods may return to the normal level (exper. 4, period 7). In experiment 5, when the intestine was washed, after each introduction of calcium lactate, the general course just described is also evident, but the figures vary too much for comparison.

In the last column of the table has been compiled the ratio of calcium to chlorine. It will be noticed that, although the concentrations of calcium used in some of the experiments present wide gaps, the ratio at which the absorption of chlorides begins to be inhibited shows a striking constancy, namely:

Experiment 2	Period 5–6,	Ca : Cl = 1 : (2.3–1.5)
Experiment 3	Period 6–7,	Ca : Cl = 1 : (1.4–0.8)
Experiment 4	Period 2	Ca : Cl = 1 : 1.0
Experiment 5	Period 4	Ca : Cl = 1 : 1.1

This relationship is the more remarkable when it is considered that the concentration of sodium chloride in experiments 2 and 3 is nearly twice that of the subsequent experiments. This point will receive further investigation.

In order to demonstrate the action of another calcium salt, in table 2 is presented an experiment in which the calcium was introduced in the form of calcium chloride. The following points are evident; less chlorides are absorbed from the same percentage and amount of chlorides in the form of calcium chloride than from sodium chloride. The latter was introduced into the colon in period 1 and the former in period 2. In a mixture of sodium chloride and calcium chloride (period 4), of the same chloride content, much less chlorides are absorbed than from sodium chloride alone.

TABLE 2

The effect of Ca *upon water and chloride absorption in the colon*

EXPERIMEET NUMBER	TIME	VOLUME INTRODUCED	VOLUME RECOVERED	CHANGE VOLUME FLUID − LOSS	NaCl INTRODUCED	Cl AS CaCl₂ INTRODUCED	NaCl RECOVERED	CHANGE NaCl + GAIN − LOSS	CHANGE NaCl − LOSS	RATIO Ca : Cl
	min.	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*	*mgm.*	
Experiment 1										
Period 1...	60	87	34	−53	0.33	0	0.48	+0.15	−124	
Period 2...	60	85.5	43	−42.5	0	0.34	0.54	+0.20	− 59	1:1.8
Period 3...	60	87	42	−45	0.31	0	0.45	+0.14	− 81	
Period 4...	60	85.5	43.5	−42	0.32	0.32	0.49	+0.17	− 60	1:1.0

DISCUSSION

The above data show that calcium salts affect the absorption of chlorides from sodium chloride in two stages. First: with low concentrations of calcium lactate, there is a speeding effect similar to that which we obtained with sodium sulphate on sodium chloride. Second: with still higher concentrations of the calcium lactate, the absorption of chlorides is inhibited. As might be expected, with an intermediate concentration of calcium lactate a balanced action is noted; that is, a point is reached where the chloride absorption, from the mixture of sodium chloride and calcium lactate, is of the same grade as from pure sodium chloride solution.

All of these stages may take place below a Δ iso-osmotic with the blood. On the other hand, the speeding effect of sodium sulphate persisted throughout the whole range of Δ.

When the calcium lactate is introduced with sodium chloride, there is a reversal of the normal behavior of sodium chloride of equal concentration in the colon. That is, the percentage concentration of hypotonic solutions (0.3 per cent) is reduced instead of increased. An

effect similar to that of the chloride in mixtures of sodium chloride and sodium sulphate.

There is an indication that calcium chloride behaves similarly to calcium lactate. On the basis of the ideas of Clowes (5), it is probable that the phenomena described are concerned with the predominant cation calcium and anion chlorine, an indication of which is the rather constant ratio of Ca: Cl at the point where the inhibitory action is first exerted.

There is evident in the above experiments a "lag" effect, since at one point there is an increased absorption of chlorides over the control periods which later (after the inhibitory effect) returns to about the normal level. When the colon is thoroughly washed with salt solution after each introduction of the calcium lactate this "lag" effect is still evident.

It has been shown in the preceding paper that sodium sulphate in all concentrations speeds the absorption of sodium chloride. We suggested that the effect may be related to osmotic factors alone. With calcium lactate, however, it is at once apparent that no such simple explanation is adequate. For the stage of inhibition, at least, an effect upon the permeability of the intestinal wall would seem at present to be the most probable cause of the phenomenon.

CONCLUSIONS

1. Calcium lactate in increasing concentrations first accelerates, then inhibits the absorption of chlorides from solutions of sodium chloride in the colon.

2. There is an indication that this action bears a relationship to the ratio of calcium to chlorine.

3. The first stage of the action of calcium lactate upon sodium chloride resembles that of sodium sulphate upon that salt. The last stage presents the opposite effect to that of sodium sulphate.

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AN EXPERIMENTAL CONTRIBUTION TO THE BIOLOGICAL RELATION OF THE DIASTASE-FERMENT BETWEEN THE MATERNAL BLOOD, THE FETAL BLOOD AND THE AMNIOTIC LIQUID OF ANIMALS

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As to the questions whether the change, natural or artificial, produced in the maternal blood has any influence upon the fetal blood or not, and under what law, if any, it acts upon the latter, there has been little investigation as yet. These questions, however, arouse great interest in practical medicine as the change may interfere with the embryonal metabolism, and consequently it may sometimes lead to the death of the fetus, causing an abortion or premature labor. The relation between the maternal body and the fetus and amniotic liquid means, it must be admitted, the metabolism in the placenta on one hand, and a certain function of the amniotic epithelium on the other. Few conclusive evidences have ever been given in the investigation of the function of the placenta, and accordingly the present paper will serve as an experimental proof of the general view.

That organic and inorganic substances pass into the fetal blood has been shown by experiments and published by many investigators, of which the following are the chief points that have important relations to my investigation:

Fat (which was most successfully investigated by Costa (1), Bondi (2), and Oshima (3)), has been observed to pass into the fetus not as such, but in a modified form; besides, carbohydrates (Bang (4) and Varaldo (5)), anti-bodies (Mounn af Heurlin (6)), antitoxin (Polano (7)), and haemolysine (Kreidl and Mandl (8)) are also ascertained to pass into the fetus; with regard to proteins, however, it was shown by Ascoli (9) that they do not pass through the placenta but that the latter acts as a digestive organ for them. Kreidl and Mandl's experiments, which demonstrated the passage of haemolysine into the

maternal body from the fetus, show as just stated, that haemolysine passes through the placenta.

The results of the various investigations hitherto made suggest the idea that the placenta, being an important barrier for the fetus, does not allow the free passage of anything, but serves as an alimentary and life-protecting organ of the fetus; moreover from its behavior toward glycogen (as shown by Lochhead and Cramer (10)), we may say that the fetus takes up material necessary for its development quite independently of the nutrition of the maternal body.

An interesting question which has presented itself in recent years in connection with the maternal body, the fetus and the amniotic liquid is the relation of the pigment of living bodies among these three. With regard to this question we have not yet come to a conclusive opinion, but it seems certain that coloring-matters usually do not pass through the placenta. No researches have yet been published whether a ferment will pass through the placenta and although the passage of glucose is an established fact, its quantity and its relation to the amniotic liquid have not been fully investigated by any one. I was therefore engaged in the present researches in order that I might make a contribution to the knowledge in this direction.

For my experiments I selected diastase as it is a ferment that can be accurately determined, and the object of my investigation was to know the result of the injection of diastase into the maternal body, that is to say, if the increase of diastase in the maternal blood by any means had any influence upon the fetus and the amniotic liquid. In short, it was my intention to ascertain the permeability of the placenta to the ferment. Besides, I entertained some hope that I should be able to have some glimpses of the origin of the amniotic liquid by observing its attitude toward the ferment, but to my disappointment, I could not obtain sufficient data from my experiments to throw light on the origin of this fluid. I also tried to compare the transudate in the abdominal cavity with the amniotic fluid, but as it was very difficult to obtain the latter—fair amounts may be obtained in rabbits but guinea pigs have little, if any—I excluded it from the present researches. With the above objects in view, therefore, I made the following experiments.

In the first place, I examined whether diastase would pass into the blood of the fetus by producing a retention of diastase in the maternal body by various means (such as tying the renal blood vessels and the injection of uranium).

In the second place, I examined the change of quantity of the diastase-ferment when it was artificially injected into the blood vessels, and at the same time injecting glucose, which is certainly known to pass, determined its quantity and compared it with the quantity of the ferment that passed.

In the third place, having found that when diastase was injected into the blood of an animal the greater part of it soon disappeared, I studied the injection of various other ferments in order to find the reason for the disappearance of diastase and compared these results with similar ones obtained from experiments with animals not yet impregnated.

Animals used in the experiments. Guinea pigs are best fitted for the study of diastase as its value is high in these animals, and in all my experiments I used guinea pigs fed with ordinary food, selecting bigger ones as much as possible. Of the pregnant ones I selected those which were at the period of from forty to fifty days of their pregnancy, for at an early period the fetal blood is too small in quantity, and on the other hand the amniotic liquid becomes less as the time of delivery approaches (the duration of pregnancy of the guinea pig is about nine weeks).

Methods used in drawing the blood and the amniotic liquid. The maternal blood was taken into a sterile centrifuge tube from the carotid artery. For the fetal blood, after abdominal incision the uterus was exposed and opened and the placenta separated without injury to the fetal sac. The amniotic liquid was next drained into a sterile beaker, the fetus was then carefully wiped with sterile gauze after which the fetal blood was drawn from an incision in the neck, care being taken to avoid contamination with cerebro-spinal fluid. Care must also be taken to prevent the mixture of the liquid of the extra-body cavity with the amniotic liquid, for the amniotic sac of the rabbit and the guinea pig, unlike that of man and other higher animals, retains the yolk-sac around it until the last stage of the embryonic life; for this reason it is advisable to seize the amniotic sac with the cord downward, for then the liquid will gather around the cord after which a small hole in the region of the head permits the amniotic liquid to be poured out without contamination.

Notes on the determinations of diastase. I adopted the method of Wohlgemuth (11) in determining diastase. From earlier work I found that this method was not reliable unless everything used for it was disinfected, and accordingly in my present investigation all the instruments, pipettes, beakers and test-tubes were subjected to 150°C. in the

dry heat sterilizer, and all the salt solution and starch solution were also sterilized beforehand.

The maternal blood was diluted one hundred times its volume, and the fetal blood and the amniotic liquid ten times their volumes with sterile 0.85 per cent saline solution, 1.0 cc. of each was taken into test-tubes, after which they were all again doubly diluted with common salt water. As to the starch solution, I used 0.2 per cent solution of soluble starch prepared by the Kahlbaum Company, adding 5.0 cc. to each test-tube. The tubes were carefully examined after they had been kept in a 37°C. incubator for twenty-four hours.

As an indicator a $\frac{N}{50}$ iodine-solution was used.

EXPERIMENTS ON THE DIASTASE IN THE MATERNAL BODY, THE FETUS AND THE AMNIOTIC LIQUID DURING THE RETENTION OF DIASTASE IN THE MATERNAL BLOOD

a. Determination of the diastase in the maternal blood, the fetal blood and the amniotic liquid of a pregnant guinea pig. The diastase content of the blood of the pregnant guinea pig is already known, but no one has ever determined that of the fetus and of the amniotic liquid at the same time; so I determined them preliminary to my experiments and used them as a control for my subsequent experiments. The diastase was always determined as just described. In the case of blood the serum was used for test.

The diastase value of the maternal blood in the table is from 400.00 to 800.00, the average being 602.08.

The diastase in the fetus has variable values between the two extremes of 40.00 and 322.58, the average being 109.29 for the blood and 80.31 for the amniotic fluid.

The diastase both in the fetal blood and in the amniotic liquid is remarkably small as compared with that of the maternal blood, being only one-fifth to one-sixth of the latter.

Bial (12), who investigated the diastase value in the embryonic stage of man and in the fetus of animals, also recognized a trace of diastase in the blood of the fetus. Of the ferments in the amniotic liquid we have Bondi's (13) report. According to him we have pepsin, fibrin-ferment and diastase always in the liquid, but no other ferments, and there is no doubt that we have diastase in the amniotic liquid.

b. Experiments on the change of the quantity of diastase in the maternal blood, and on its passage into the fetus after the renal blood vessels of a pregnant guinea pig have been tied. As the diastase-ferment is always present in urine, if we tie the renal blood vessels on both sides, it is probable that a retention of diastase would occur in the maternal body. To test this point the following experiment was performed on a pregnant guinea pig with particular reference to the diastase content of the fetus. Under light ether anesthesia the kidneys were exposed by dorsal incisions. Ligatures were tied about the artery, vein and ureter on either side and the abdominal incision closed. The procedure occupied less than five minutes. In no case did the animal live more

TABLE 1

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	DIASTASE OF THE		
			Maternal blood	Fetal blood	Amniotic liquid
	grams				
1	490	About 50	D=416.66		
2	580	About 50	400.00	D=40.00	
3	620	More than 40	400.00	80.00	
4	850	More than 50	800.00	161.28	
5	900	More than 40	800.00	40.00	D= 40.00
6	720	More than 40	400.00	40.00	40.00
7	700	More than 50	800.00	322.58	80.00
8	680	More than 50	800.00	80.00	161.29
Average.....			602.082	109.122	80.31

than twenty-four hours, and many of the feta were found dead when observations were made three to six hours after the operation.

Diastase was determined by the same method as before. The diastase content of the maternal blood varies from 800.00 to 3225.80, averaging 1250.177.

When compared with those of the control (table 1), we see that the minimum here corresponds with the maximum of the control and that the maximum is four times as great after ligation of the renal vessels than normally. Besides, the individual cases in the table evidently show an increase of diastase in the maternal blood and that the diastase content in the fetal blood, which is 80.00 to 322.58, with the average of 161.13, is somewhat greater than 109.122, which is the average diastase value of the fetus in the control experiments. If no. 7

in the control series, which deviates widely from the average, be omitted the average becomes 73.54. Using this figure as the control value the ligation experiments show a twofold increase in fetal blood diastase. It appears therefore that the increase in the diastase content of the maternal blood as the result of ligation of the renal vessels is responsible for the increase in diastase content of the fetal blood.

In the present experiments I had six cases of dead fetus, all of which were examined shortly after their death. The relation between death and the quantity of diastase may depend upon the time that elapses after the death, and in my small number of experiments I found that there was little relation between them, that is to say, in some feta

TABLE 2

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	TIME AFTER OPERATION	DIASTASE OF THE		FATE OF FETAL LIFE
				Maternal blood	Fetal blood	
	grams					
1	700	More than 50	6°0′	D=800.00	D=161.29	Dead
2	750	More than 50	5.40	800.00	161.29	Dead
3	640	More than 50	4.0	1612.90		Dead
4	760	More than 50	3.40	3225.80	322.58	Living
5	760	More than 50	3.30	800.00	80.00	Dead
6	800	More than 50	3.0	800.00	161.29	Dead
7	700	More than 40	3.0	800.00	80.00	Living
8	740	More than 50	4.0	800.00	161.29	Living
9	700	More than 50	4.0	1612.90	161.29	Dead
Average.				1250.177	161.13	

still alive I found little diastase while in some dead ones there was a large quantity of it, and thus it pointed to the negation of any definite relation between them. The operation of tying the blood vessels of the kidneys must be a very great stimulus and it is possible that such a stimulus might cause the increase of the ferment. However since diastase determinations made shortly after the operation was completed never showed an increase over the control and usually showed a decrease, I believe this factor is of no significance.

c. The relation of diastase in the case of nephritis caused by the injection of uranium. It is known that the injection of uranium gives rise to nephritis, producing a lesion in the convoluted tubule in particular. I made this experiment in order to see if nephritis produces any change

in the quantity of diastase in the maternal blood or in the fetal blood and amniotic liquid.

Treatment. From three to eight days after the subcutaneous injection of a 0.1 per cent solution of uranium acetate into the abdominal wall of the animal in the proportion of 1 cc. per kilogram of the body weight, I took the blood and the amniotic liquid and examined them. Before the experiment I always ascertained the presence of albumin in the urine (albuminuria) and measured its quantity by Sueyoshi's (14) albuminometer.

The diastase in the maternal blood is 800.00 to 6250.00, averaging 1901.61. No. 3, however, deviates widely from the average. If this

TABLE 3

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	TIME AFTER INJECTION	QUANTITY OF ALBUMIN	DIASTASE OF THE			STATE OF FETAL LIFE
					Maternal blood	Fetal blood	Amniotic liquid	
	gms.		*days*	*per cent*				
1	680	More than 50	4	1.5	D=3225.80	D=322.58		Dead
2	700	More than 40	2	2.0	1612.90	Dead		Dead
3	580	More than 50	3	3.0	6250.00	Dead	D=80.00	Living
4	900	More than 50	3	1.5	1612.90	161.29		Living
5	860	More than 50	3	0.5	800.00	80.00		Living
6	740	More than 50	4	0.5	1612.90	322.58		Living
7	770	More than 50	4		800.00	80.00	40.00	Living
8	720	More than 50	8	2.0	800.00	40.00	80.00	Living
9	840	More than 50	4	0.5	400.00	161.29	80.00	Living
Average.					1901.61 (1736.08)	166.82	65.00	

determination be omitted, the average becomes 1736.08. Even this average shows a great increase when compared with that of the control.

The diastase in the fetal blood is 80.00 to 322.58, average 166.82, thus showing a slight increase as in the preceding case. The diastase value of the amniotic liquid being 40.00 to 80.00, averaging 65.00, shows no noteworthy tendency of increase.

As indicated in the table (table 3), the minimum of the diastase value of the maternal body corresponds with the highest of the control, and the maximum is seven times as large as that of the latter. The individual cases all clearly show an increase, indicating that there is a retention of diastase in the maternal body. In the fetus we find some

increase, but there is no influence whatever upon the amniotic liquid. If there is any relationship between the quantity of diastase retained and the quantity of albumin or the length of time after the injection, my small number of experiments failed to show it.

From the two preceding experiments, we may conclude that when diastase is retained in the maternal blood, the diastase ferment passes into the fetal blood. There is, however, no evidence of its passage into the amniotic liquid. This indicates the passage of the ferment through the placenta and is undoubtedly dependent upon the physical permeability of the organ.

THE DIASTASE AND THE BLOOD-SUGAR IN THE MATERNAL BLOOD, FETAL BLOOD AND AMNIOTIC LIQUID AFTER AN ARTIFICIAL INJECTION OF DIASTASE AND GLUCOSE

a. The determination of the blood-sugar in the maternal blood, fetal blood and amniotic liquid of a pregnant guinea pig. The determination of sugar in the maternal blood, fetal blood and amniotic liquid of a

TABLE 4

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	SUGAR OF THE		
			Maternal blood	Fetal blood	Amniotic liquid
	grams		*g/dl.*	*g/dl.*	*g/dl.*
1	900	More than 40	0.1110	0.0633	0.0751
2	720	More than 40	0.0869	0.0462	0.0820
3	700	More than 50	0.1099	0.1199	0.0655
4	680	More than 50	0.1435	0.0433	0.0767
Average.....			0.11282	0.06817	0.07432

pregnant guinea pig has not been reported. This is probably due to the fact that the quantity of the fetal blood and the amniotic liquid is so small that the method of determining sugar hitherto in use is impracticable with them.

Bang's (15) micro-method being best suited for the present investigation, I soaked four pieces of blotting-paper prepared after Bang's method in the bloods, and two other pieces in the amniotic liquid. The diastase was determined as before. For the determination of sugar I used potassium chloride with acidity, which was Bang's original method, and for titration a $\frac{N}{200}$ iodine solution.

The blood-sugar of the maternal blood is 0.0869 to 0.1435 g/dl., averaging 0.11282 g/dl.

The blood-sugar of the fetus is 0.0462 to 0.1199 g/dl., averaging 0.06817 g/dl.

The sugar in the amniotic liquid is 0.0655 to 0.0767 g/dl., averaging 0.07432 g/dl.

The quantity of sugar in the maternal blood, like that of man and other animals, seems to be of the value of about 0.1 g/dl. but in the fetus and in the amniotic liquid it is very much less.

TABLE 5

NUMBER	BODY WEIGHT	DAYS OF PREGNANCY	QUANTITY OF INJECTION	CONCENTRATION OF SOLUTION OF DIASTASE	TIME OF BLOOD DRAWING	DIASTASE OF			SUGAR OF		
						Maternal blood	Fetal blood	Amniotic liquid	Maternal blood	Fetal blood	Amniotic liquid
	gms.		*cc.*	*per cent*							
1	1070	More than 60	1.0	1.0	15′–20′	D= 200.00	D= 161.29		0.1677	0.0991	
2	900	More than 55	1.0	1.0	15′–20′	400.00	161.29		0.1820	0.1092	
3	770	More than 55	1.0	1.0	20′–25′	400.00	161.29		0.1132	0.1433	
4	950	More than 50	3.0	1.0	20′–25′	800.00	80.00		0.2019	0.0595	
5	900	More than 50	2.0	0.5	20′–25′	800.00	80.00	80.00	0.1572	0.1133	0.0715
6	1200	More than 50	4.0	0.5	20′–25′		161.29	161.29	0.1094	0.1045	0.0783
7	800	More than 50	4.0	0.1	30′–35′	800.00	80.00	322.58	0.1895	0.1411	0.0567
8	900	More than 50	7.0	0.1	20′–25′	400.00	80.00	161.29	0.2006	0.1713	0.0530
9	740	More than 50	3.0	0.5	5′	800.00	80.00	80.00	0.3357	0.1174	0.0558
10	650	More than 50	3.0	0.5	5′	800.00	161.29	80.00	0.2120	0.0821	0.0782
11	690	More than 50	3.0	0.5	5′				0.2536	0.1499	0.0461
12	720	More than 50	3.0	0.5	5′				0.2712	0.1711	0.0499
Ave.						600.00	121.895	147.29	0.19950	0.13015	0.06118

b. The diastase and the sugar in the maternal blood, fetal blood and amniotic liquid after the injection of Taka-diastase and glucose. I made the following experiments in order to study the influence of a ferment upon the maternal body, the fetus and the amniotic liquid when it was injected into the maternal body parenterally and also to compare the result with that of a substance which is already known to pass. I used Taka-diastase for the ferment and glucose (Merck) for the substance with which to compare it.

Method of experiments. I dissolved Taka-diastase in the proportion of 0.1, 0.5 and 1 per cent in a 0.85 per cent salt solution, and after neu-

tralizing it with a $\frac{N}{10}$ soda solution, added pure glucose in the proportion of 10 per cent. This solution was injected into a pregnant guinea pig through the vein on one side of the neck with Pravatz's injector under a very slight pressure, for when the solution is quickly injected, it causes various disorders in the body of the animal, but if it is injected very slowly, the animal is able to stand a large quantity of it in considerable concentration. The blood of guinea pigs injected with different quantities of the solution is taken at intervals of time for the determination of the diastase and glucose in the maternal blood, in the fetus and in the amniotic liquid. The table shows that the diastase of the maternal blood is from 200.00 to 800.00, averaging 600.00. The diastase of the fetus is 80.00 to 161.29, the average being 120.64. The diastase of the amniotic liquid is 80.00 to 322.58, averaging 147.52. The blood-sugar of the maternal body is 0.1094 to 0.3357, averaging 0.19950 g/dl., that of the fetus is 0.0595 to 0.1713, the average being 0.13015 g/dl.; that of the amniotic liquid is 0.0567 to 0.0783, averaging 0.06128 g/dl. Thus although a diastase-ferment is artificially injected into the blood stream, no change of quantity of diastase takes place in the maternal blood.

In the fetal blood, however, the average shows a slight increase, and when we look at the individual cases, we find that the most of them fall close to 161.29, which shows an increase of diastase in the case of the fetal blood. In the case of the amniotic liquid there is also an apparent increase of diastase.

Sugar increases both in the maternal and the fetal blood, and since we see that both diastase and sugar, even in the case of an artificial parenteric injection, produce its increase in the fetal blood as in the case of the autoretention, it is certain that the ferment, like sugar, passes through the placenta.

In the amniotic liquid we recognize a little increase of the ferment, but no passage of sugar into it. This fact is difficult to explain.

In the case of an artificial introduction of diastase into the body, unlike the case of the autoretention of the ferment, I found a singular phenomenon in that there was no increase of diastase in the maternal blood even though a pretty large quantity of diastase was injected into it. There are only three possible explanations for this phenomenon: *a*, a regulation may take place in the placenta and the diastase in the maternal blood may be always adjusted by it; *b*, the ferment may soon disappear on account of its toxic influence even if it be introduced

in large quantity into the body; or *c*, it may be due to a regulation that may take place in some part of the maternal body.

I shall again touch these questions in the following chapter. As regards the concentration and the quantity of the solution and the length of time that intervenes between the injection and the drawing of blood, I may say that they have no relation to the quantity of diastase though my experiments are few in number.

When sugar is passed directly into the vein, unlike the sugar that is absorbed by the alimentary canal, it is not affected by any digestive ferment or by the action of the liver, but enters the system of the general circulation directly. According to Bang's (15) experiments, the greater part of the injected glucose undergoes transformation and disappears at once while a part of it is deposited in various organs or is excreted by the kidneys, and for this reason the glucose that appears in the measurement is very small in quantity when compared with the injected glucose which is as much as 10 per cent. According to Bang, moreover, the blood-sugar reaches the acme of its quantity five minutes after its intravenous injection and for thirty minutes after that it gradually decreases. It was this observation which led me to select the time shown in the table for drawing the blood in my experiments.

As regards the question whether the quantity of injected grape-sugar has anything to do with blood-sugar, my experiments are too few to settle it.

DETERMINATION OF DIASTASE AFTER THE INJECTION OF TAKA-DIASTASE, TRYPSIN AND PEPSIN

We have learned from the preceding experiments that the diastase in the maternal blood does not increase if we introduce it artificially into the maternal body, and I have made some suggestions to explain this fact. Here I propose to take up the question whether or not the diastase is affected by the placenta, and also the possibility that the diastase originally present in the blood may be destroyed by the toxic influence of the added ferment, the injected diastase replacing it so that the circulating diastase remains unchanged. The third supposition that the diastase may be directly adjusted by a regulation that may be taking place in some part of the maternal body is a complex question and will not be considered at the present time.

According to Oppenheimer (16), ferments exert little toxic influence on the body, while Hildebrandt (17) supposes that the toxic influence

is due to the albumose or the amino-acid, etc., contained in it. Wishing to see if ferments really had no toxic influence, I injected various ferments into animals and observed the change of the blood-diastase, for the presence or absence of a toxic influence can be inferred from the change produced on the diastase. The guinea pigs used in the present experiments were all non-pregnant ones, for if the injection of ferments into non-pregnant animals produces the same change as in a pregnant one, it will at once prove that the change has nothing to do with the placenta.

The ferments used in the present experiments were Taka-diastase, trypsin (prepared by the Grübler Company), and pepsin (Sankyo Company).

It was necessary therefore to establish a control for this new technique. The results obtained by this method are given in table 6.

a. Determination of the blood-diastase of non-pregnant guinea pigs. In the following experiments, unlike the foregoing determination of diastase, the serum was not diluted and the starch solution was of 1.0 per cent.

TABLE 6

NUMBER	BODY WEIGHT	DIASTASE
	grams	
1	450	D=312.50
2	450	312.50
3	500	625.00
4	550	312.50

The blood-diastase of non-pregnant guinea pigs varies between 156.25 and 625.00.

b. Determination of diastase after the injection of Taka-diastase. For injection a 0.5 per cent solution of Taka-diastase in physiologic salt solution neutralized with a $\frac{N}{10}$ soda solution was prepared. This was injected into the vein as before, and the difference of the diastase value was likewise observed by drawing the blood at different times and after the injection of different quantities. The method of determination was the same as before.

The blood-diastase of the animals injected with Taka-diastase shows no difference from that of the ordinary animal, and it is also quite independent of the quantity injected and of the time of drawing the blood.

TABLE 7

NUMBER	BODY WEIGHT	CONCENTRATION OF SOLUTION	AMOUNT OF INJECTION	TIME OF BLOOD DRAWING	DIASTASE	CONDITION AFTER THE INJECTION
	grams	*per cent*	*cc.*			
1	340	0.5	2.0	1′	D=312.50	No particular symptoms of poisoning
2	400	0.5	4.0	Immediately after	312.50	No particular symptoms of poisoning
3	300	0.5	3.0	2′	312.50	No particular symptoms of poisoning
4	360	0.5	3.6	5′	156.25	No particular symptoms of poisoning
5	400	0.5	4.0	10′	312.50	No particular symptoms of poisoning
6	380	0.5	3.8	30′	312.50	No particular symptoms of poisoning

TABLE 8

NUMBER	BODY WEIGHT	CONCENTRATION OF SOLUTION	AMOUNT OF INJECTION	TIME OF BLOOD DRAWING	DIASTASE	CONDITION AFTER THE INJECTION
	grams	*per cent*				
1	380	1.0	0.95	5′	D=312.50	No particular symptoms
2	340	1.0	1.7	5′	312.50	No particular symptoms
3	350	1.0	3.5	5′	625.00	No particular symptoms
4	420	1.0	3.2	5′	312.50	No particular symptoms
5	320	2.0	3.2	5′	625.00	Uneasy

TABLE 9

NUMBER	BODY WEIGHT	CONCENTRATION OF SOLUTION	AMOUNT OF INJECTION	TIME OF BLOOD DRAWING	DIASTASE	CONDITION AFTER INJECTION
	grams	*per cent*				
1	360	1.0	1.8	5′	D=400.00	No particular symptoms
2	360	1.0	3.6	5′	800.00	No particular symptoms
3	380	1.0	3.8	5′	312.50	No particular symptoms
4	360	2.0	3.2	5′	312.50	No particular symptoms
5	420	2.0	4.2	5′	161.29	Uneasy, palpitation of the heart, suspension of respiration, blood-flowing, dark color

c. Determination of diastase after the injection of trypsin. The experiment was carried on in the same manner as the preceding, and the solution of trypsin was of 1.0 to 2.0 per cent. Though I found in the preceding experiment that the time of drawing the blood and the quantity injected had little relation to the blood-diastase, I injected different quantities of trypsin as shown in the table, with the hope that different kinds of ferments might produce different results. The trypsin was also neutralized with a $\frac{N}{10}$ soda solution.

The result of the injection is the same as the preceding, being quite independent of the quantity injected and showing no difference from that of the ordinary animal.

d. Determination of diastase after the injection of pepsin. The concentration of the pepsin liquid (a solution of pepsin in physiologic salt solution) was from 1.0 per cent to 2.0 per cent. After neutralizing it with a soda solution as before, it was injected into the blood vessel in the proportion of 0.5 to 1.0 per kilo.

As in the two preceding cases, the quantity of diastase remains unchanged, being quite independent of the concentration of the pepsin-liquid and the quantity injected.

Experiments were also made by injecting larger quantities of these ferments, but in such cases the animals either died or presented serious symptoms, and accordingly they are all excluded from the tables as it is not proper to take the quantities of diastase of such cases into account.

According to Hildebrandt (17), the lethal quantity of diastase, pepsin etc., for a rabbit of medium weight is 0.1 gram, and according to Ishihara's (18) experiments, if more than 0.07 gram of trypsin is injected in the vein, anaphylactic shock is produced in two cases out of three. In my experiments therefore the quantity of ferments injected into the animal body was kept within such limits that it would not produce these symptoms, and only those animals which presented such slight symptoms as palpitation of the heart, a little difficulty of respiration, etc., as given in the tables, were subjected to the examination.

As we see in the present experiments, the diastase introduced parenterally into the body also disappears at once even in the cases in which the placenta is not concerned, which shows that it has nothing to do with the placental regulation. Nor can we consider it due to a toxic influence of the ferment, for if there is such an influence we should expect a noticeable change in the diastase in the experiments. The disappearance, therefore, of the diastase parenterally injected into the

animal body without producing any increase of the blood-diastase must be explained by the supposition that there is an arrangement in the body by which the injected diastase is destroyed at once.

According to Hildebrandt's investigation, the ferment is a blood-toxin which reduces haemoglobin. We cannot, however, tell the fate of the ferment until we settle the question whether it, like sugar, disappears at once or is taken up by the liver, pancreas, muscles, etc., or whether it is destroyed by some glandular secretion or is excreted in urine, and as it is not directly connected with my present experiments nor has my investigation of it yet come to completion, I will not deal with it here.

SUMMARY

1. When the blood vessels of the kidneys are tied or when nephritis is caused by the injection of uranium, a retention of diastase in the maternal blood is produced and there is an increase of diastase in the fetal blood.

2. In the case of nephritis after the injection of uranium, there is no change in the quantity of diastase in the amniotic liquid.

3. When Taka-diastase and pure glucose are injected simultaneously into the vein, blood-sugar increases both in the maternal and fetal bodies, but no change is produced in the amniotic liquid and there is no change in the diastase of the maternal blood, but we find a little increase of it in the fetus and in the amniotic liquid.

4. From the above facts we are entitled to say that the placenta is permeable to the diastase-ferment.

5. We cannot explain the reason why the diastase-ferment passes into the amniotic liquid while sugar does not.

6. In the case of the parenteral injection of a ferment it is lost in the animal body, which seems to be due not to the toxic influence of the ferment or to the regulation of the placenta, but to the regulating function of some other part of the body.

7. As regards the origin of the amniotic liquid, there are many complicated relations, and it is difficult to explain it by the simple results of the present experiments.

In concluding this paper I wish to express my deep thanks to Professor Doctor Katayama, who kindly permitted me to pursue my investigation in his department, and to Professor Doctor Mita, who kindly guided and advised me throughout this investigation.

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THE SYNERGY AND ANTAGONISM OF SODIUM SALTS IN BARIUM STIMULATION

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The stimulating power of the sodium salts and the necessity of these salts for the activity of most contractile structures have long been recognized. Ringer (1) observed the rhythmical contractions of a muscle placed in a sodium chloride solution. Lingle (2), (3) showed that without sodium salt the heart beat is impossible, and the rhythmical activity of the jellyfish was by Loeb (4) also ascribed to this salt.

Concerning the stimulating effect of barium chloride Ringer (1) noted that its power to produce contractions and persistent spasm much exceeds that of the saline solutions and that the previous application of a saline solution increases the stimulating effect of subsequently applied barium. Overton (5) found that a muscle which had been treated with a 6 per cent cane sugar solution for a sufficient length of time failed to respond to barium stimulation; he attributed this to the lack of sodium salts in the sugar-treated muscle. This I (6) corroborated by showing that if a muscle is bathed with a 6 per cent cane sugar solution for twenty minutes, the subsequent application of m/80 barium chloride in 6 per cent cane sugar produced a few feeble contractions only after a latent period of eighteen minutes; a normal muscle in the same concentration of barium chloride in a m/8 sodium chloride solution begins to show violent twitches in from one to five minutes. Such a sugar-treated muscle placed in a 0.7 per cent sodium chloride solution speedily regains its normal irritability toward barium. The loss of irritability which a tissue experiences during its stay in a solution of a non-electrolyte is generally said to be due not so much to the specific action of the particular non-electrolyte as to the diffusion of the necessary salts from the irritable structure into the non-electrolyte (7). Hence, the presence of sodium salt in the muscle is indispensable for the stimulating action of barium and the introduction of more sodium chloride into the muscle may favor this stimulation.

While working on the stimulation of a muscle by the outward diffusion of previously introduced barium salts, I had frequent occasion to observe the strong stimulation of the muscle when it was removed from the barium solution. In 1901 Loeb (8) discovered the curious phenomenon that a muscle may be perfectly quiescent in a certain solution but is thrown into violent twitches by the removal of the solution, these contractions ceasing almost immediately upon replacing the muscle in the original solution. The solutions which Loeb found capable of causing this reaction are, with one exception, the salts of sodium which cause precipitation or inactivation of calcium, such as the oxalate, fluoride, tartrate and citrate of sodium. This most unusual behavior of the muscle Loeb called contact reaction and the form of irritability developed in the muscle by the above-named reagents he designated as "contact irritability." Subsequently I (9) showed that it is not necessary to precipitate the calcium in the muscle in order to establish this contact irritability for the previous bathing of a muscle with a solution of the chloride of rubidium or caesium enables various other salts of sodium, lithium and ammonium to call forth contact reactions.

When the gastrocnemius muscle of a frog is placed in a solution composed of 10 cc. 0.2 m dextrose plus 3 drops of m/8 barium chloride, nothing may happen for one or two hours; but the moment the solution is removed, the muscle goes into contractions which cease when it is brought back into the solution. In figure 1 the muscle, previous to *a*, has been in the above named solution for thirty minutes; at *a* the solution is removed and as a result, the hitherto quiet muscle is thrown into a strong contraction; the replacing of the barium chloride and cane sugar solution at *b* quiets the muscle instantly. At *c* the removal of the solution once more causes activity on the part of the muscle. When the barium chloride is diluted with a 0.7 per cent sodium chloride solution instead of 6 or 7 per cent cane sugar, no contact reaction takes place. A muscle in 10 cc. m/8 NaCl plus 2 or 3 drops m/8 $BaCl_2$ shows, after a latent period varying from two to thirty minutes, energetic twitches as long as the muscle remains in the solution; on removing the muscle from the solution the twitches cease very speedily, to begin again on replacing the muscle in the solution.

The behavior of a muscle placed in a m/420 $BaCl_2$ when this is diluted with 7 per cent cane sugar is, therefore, very different from that of a muscle bathed with m/420 $BaCl_2$ in 0.7 per cent NaCl. In the first solution the muscle remains quiet for an almost indefinite period,

but instantly goes into strong contraction when it is removed from the solution; in the second case the muscle is soon thrown into twitches of greater or lesser violence which cease very soon after the solution is removed. In other words, barium in the presence of sodium stimulates as long as it is in contact with the muscle while in the presence of sugar barium acts only on the removal of the muscle from the solution. The reason for this difference lies in the influence of sodium salts on the twofold action of barium. This twofold action of barium consists of: first, what I shall call the "direct" or ordinary stimulating effect when a muscle is immersed in a solution of a barium salt; second, aside from this usual stimulating action which barium shares with many other chemical compounds, barium calls forth, under the proper

Fig. 1. At *a* and *c* the muscle is removed from a solution composed of 10 cc. 0.2 m dextrose + 3 drops m/8 $BaCl_2$. At *b* the solution is replaced. At *d* 3 drops of m/8 NaCl are added to the above solution; at *c* the muscle is removed. At *f* the original solution used at *a* is replaced and at *g* the muscle is removed from it.

conditions, a contact reaction which is decreased or altogether inhibited by many of the sodium salts.

This action of sodium in retarding the development of contact reaction by barium can be proved in various ways:

1. While m/420 barium chloride in 7 per cent cane sugar gives rise to contact reaction in ten or twenty minutes, this same concentration of barium in 0.7 per cent sodium chloride has no such effect.

2. In figure 1 the muscle bathed with 10 cc. 0.2 m cane sugar plus 3 drops m/8 $BaCl_2$ gives rise to contact reaction shown at *a* and *c*; at *d* the muscle is returned to the solution after three drops of m/8 sodium chloride have been added. Eleven minutes later, at *e*, the muscle is again exposed to the air but, it will be seen, no contact reaction takes place. At *f* the original solution of barium chloride in cane sugar is

replaced and, after seven minutes, removal of the muscle from the solution, at *g*, once more causes contact reaction.

3. The two gastrocnemius muscles of a frog were prepared; one was placed for twenty minutes in m/8 sodium chloride; the other, in 7 per cent cane sugar for the same length of time. At the expiration of this period, both muscles were immersed in 10 cc. 7 per cent cane sugar plus 3 drops $BaCl_2$; the muscle previously bathed with cane sugar showed contact reaction in fifteen minutes while the muscle treated with sodium chloride did not for thirty-five minutes. It is evident that the removal of sodium salts from the muscle previous to the application of barium favors the development of contact irritability.

4. This antagonism of sodium to barium can also be demonstrated in the following manner. The addition of 1 or 2 drops of sodium chloride to a solution of cane sugar and barium chloride in which the muscle has developed contact reaction very promptly abolishes the reaction; if now a few more drops of barium chloride are added, the muscle again acquires contact irritability.

5. If the sodium chloride is added immediately to the barium solution the usual contact reaction does not appear; thus, in 10 cc. 7 per cent cane sugar plus 3 drops m/8 $BaCl_2$ plus 5 drops m/8 NaCl the muscle did not develop contact irritability although it was allowed to remain in the solution for more than an hour.

What has been said concerning the antagonistic influence of the chloride of sodium also applies to the bromide, iodide, nitrate, nitrite and chlorate; this is somewhat surprising for most of the above-named salts themselves stimulate the muscle-nerve preparation, being in this respect far superior to the sodium chloride. Mathews (10) gives the order of increasing power to stimulate the motor nerve as:

$$NaCl < NaBr < NaI.$$

I (11) found that while the gastrocnemius muscle may remain for two or three hours in a m/8 sodium chloride without any contractions appearing, in a m/8 sodium iodide or bromide solution the muscle is very soon thrown into violent twitches. Hence, we have here an example of an agent which in itself stimulates greatly but which inhibits the stimulation brought about by another agency. A few experiments with sodium acetate and citrate gave opposite results to those obtained with the iodide, bromide, etc., but this will be detailed in a future article. The chloride of potassium, calcium, lithium and magnesium all abolish the contact irritability established by barium salts. Also

may it be stated that barium nitrate behaves in same manner as the chloride.

The production of contact irritability by a solution containing cane sugar and a barium salt is due primarily, I think, to the barium. A cane sugar solution having an osmotic pressure equivalent to that of the muscle—a 0.2286 mole, according to Webster (12)—does not cause contact reaction; even a 0.35 m cane sugar and a 0.8 m dextrose solution fail to do so, even though they are applied for one or two hours. Whether the sugar solution plays any other part than to serve as a diluent for the barium salt and as an indifferent fluid which allows the sodium salts to diffuse out of the muscle has not been definitely ascertained. Neither have I gained any further knowledge as to the external condition that causes a muscle to go into contraction on its removal from a solution in which it had remained at rest for an hour or more. This investigation concerns itself merely with the striking fact that barium has a twofold action on the muscle-nerve preparation; first, a direct stimulation and, second, the development of contact irritability. The first of these effects of barium is not only favored by sodium salts but absolutely depends upon the presence of these salts. This acceleration is especially true for such salts of sodium as the bromide and iodide which in themselves stimulate the muscle and which in the presence of barium add their effects to that of barium; they may therefore be regarded as synergistic agents. On the other hand, these same salts of sodium antagonize the action of barium in establishing contact reaction.

This peculiar behavior of sodium salts is not due to any action upon the motor nerve as the following experiments prove. The sciatic nerve of a muscle-nerve preparation was placed in a solution of 10 cc. m/8 NaCl plus 3 drops of m/8 $BaCl_2$ for one or two hours; judged by the condition of the muscle, this solution failed to stimulate the nerve. When the muscle is placed in this concentration of barium chloride, it goes into twitches in from two to ten minutes. During a brief stay in the dilute barium chloride the nerve is not injured, for its irritability, as measured by the strength of faradic current necessary to stimulate, is actually greater thirty or forty minutes after its immersion in the barium solution than before. However, this may be only an apparent increase in irritability, due, as Shōji (13) showed, to the increased electrical conductivity caused by the passing of the salt into the nerve. Neither is a nerve stimulated by m/420 $BaCl_2$ in a 0.2 m cane sugar or dextrose solution, nor is the removal of the nerve from this solution

followed by any activity on the part of the muscle, i.e., no contact irritability is established. It is therefore highly probable that these two effects of barium salts are brought about in the muscle itself.

The explanation of these two effects of barium and the synergistic action of sodium salts in the usual form of stimulation by barium and their antagonistic action in the production of contact reaction, no doubt, depends upon the explanation that must still be found for the contact phenomenon in general. Meanwhile two explanations suggest themselves. It is conceivable that barium in the presence of sodium stimulates a certain structure in the muscle and thereby causes the muscle to exhibit the usual contractions observed as long as the muscle remains in the solution. But, in the absence of sodium, barium merely so alters this structure that it readily responds to a new (or additional) form of stimulus, the nature of which is at present unknown and which becomes operative only when the muscle is removed from the solution; the change brought about by barium in this irritable structure is prevented by sodium salts. Or we may presuppose two irritable structures in the muscle, e.g., the muscle fiber itself and the receptive substance. One of these is acted upon synergistically by sodium and barium; this leads to what we have called the direct stimulation. The other structure is so affected by the barium that contact irritability is established and the establishing of this contact irritability is prevented by sodium. Both these actions of barium are antagonized by potassium, calcium and magnesium ions.

SUMMARY

1. Barium salts have two effects upon the muscle:

a, They cause "direct stimulation" which results in contractions of the muscle as long as the muscle remains in the solution.

b, They establish "contact irritability" which the muscle exhibits only when it is removed from the solution.

2. For the "direct stimulation" by barium the presence of sodium salts is necessary; removal of these salts by immersing the muscle in an isotonic cane sugar or dextrose solution renders this stimulation impossible.

3. In the establishing of "contact irritability" by barium, certain sodium salts act antagonistically and therefore their removal by bathing the muscle in a sugar solution is necessary.

4. Both the direct stimulation and the contact reaction are antagonized by potassium, calcium and magnesium.

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DISTRIBUTION OF THE ANTINEURITIC VITAMINE IN THE WHEAT AND CORN KERNEL

A Contribution to the Biology of the Antineuritic Vitamine

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The work of the last ten years on the presence of the antineuritic vitamine in foodstuffs has conclusively demonstrated that the seeds of the ordinary cereals are relatively rich in this accessory food. The study of beriberi, for instance, has shown that the whole rice kernel is a prophylactic against this disease, whereas highly milled rice is devoid of this property. The difference in the two kinds of rice is, that the highly milled variety does not contain the peripheral layers nor the germ of the seed, these portions being removed with the "offal" in the milling process. It was found, furthermore, that the rice offal (polishings) contain the antineuritic vitamine, and the question then arose as to the location of the substance in the peripheral layers of rice. Funk (1) and many others claimed that the seat of the vitamine was to be found in the aleurone cells. This claim was, however, never substantiated by experimental evidence, but it nevertheless led to a similar doctrine regarding the distribution of the antineuritic vitamine in the wheat and corn kernel.

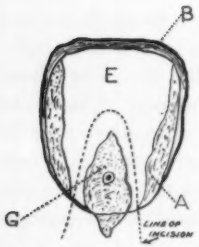
In 1915 McCollum and Davis (2) were able to show that the wheat embryo is more efficient in promoting growth than the entire wheat. It should be stated that these authors used as wheat germ a product which was obtained from a flour mill and which, although being largely composed of the embryo of wheat, was contaminated by other portions of the grain. About the same time Voegtlin, Sullivan and Myers (3) demonstrated that commercial corn germ has prophylactic value in avian polyneuritis.

A little later, extensive investigations by Voegtlin and Myers (4) on the relative food value of various wheat flours and corn foods, brought

out the fact that those of the modern mill products which were particularly rich in embryo, of either corn or wheat, were also very effective in the treatment and prevention of the polyneuritis of pigeons.

During the progress of the present investigation an article appeared by Chick and Hume (5) in which these authors also call attention to the germ of cereals as being the chief seat of the antineuritic substance. They consider the aleurone layer as next in importance in this respect.

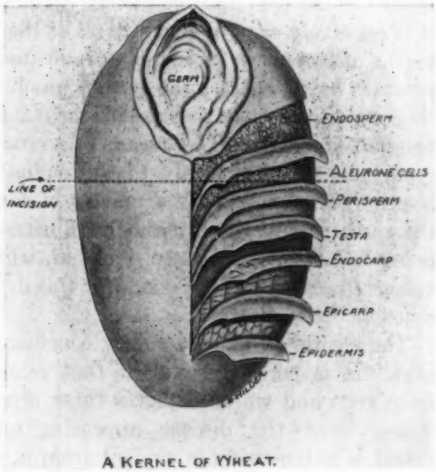
From the foregoing, it is evident that the embryo of corn and wheat is probably the part of the seed which contains a considerable amount of antineuritic vitamine. This conclusion is fairly well justified



CROSS SECTION OF CORN KERNEL

A = ALEURONE LAYER.
B = BRAN.
E = ENDOSPERM.
G = GERM (EMBRYO).

Fig. 1



A KERNEL OF WHEAT.

Fig. 2

although, as has already been pointed out, the ordinary commercial wheat or corn germ does not contain the histological elements of the embryo only, but also some bran and endosperm. For theoretical and practical reasons it was very important to follow out this line of investigation with a view to decide the following questions:

Is the antineuritic vitamine located in both the aleurone layer and the embryo, or only in one of these tissue elements?

The relations of the various parts of the corn and wheat kernel are illustrated by figures 1 and 2. The research was carried out on adult pigeons, series of which were fed on the following mixtures:

1. "Degerminated" corn, corn kernel from which the germ had been removed.
2. "Degerminated" corn, 90 per cent, casein (purified), 5 per cent, salt mixture, 5 per cent.
3. "Degerminated" corn, 85 per cent, casein (purified), 5 per cent, salt mixture, 5 per cent, butter fat, 5 per cent.
4. Whole corn.
5. "Degerminated" wheat.

In regard to the removal of the germ, it should be emphasized that this has to be carried out with painstaking accuracy, as otherwise the results of the feeding experiments are misleading. In the case of corn it is relatively easy to separate all of the germ from the endosperm by means of two sharp incisions, as indicated in figure 1. The corn endosperm is hard, whereas the germ is much softer, a fact which facilitates their separation. In the case of wheat it is necessary to sacrifice nearly one-half of the kernel by means of a cross-section (fig. 2). The wheat germ adheres very firmly to the endosperm and is very difficult to remove completely. As a matter of fact, we are convinced that in the early part of the experiment, illustrated by chart 5, not all of the germ had been completely removed, which explains the delay in the onset of polyneuritis. Soon after this defect was remedied the pigeons developed the disease.

The results obtained are very conclusive and need very little discussion. It is perfectly evident that removal of the germ or embryo from corn and wheat deprives these cereals of their antineuritic vitamine. That the disease appearing on a diet of "degerminated" cereal is polyneuritis is evident from *a*, the typical symptoms; *b*, the histological changes in the sciatic nerve; and *c*, the fact that the administration of an alcoholic extract of corn germ or autolyzed brewer's yeast leads to a prompt disappearance of the symptoms. It is, furthermore, interesting to note the rapid recovery of body weight and well-being following a change to whole corn or wheat, respectively. Proof that the symptoms are not due to the deficiency of the "degerminated" cereal in other essential foods is furnished by the experiments illustrated by charts 2 and 3. Chart 4 and other unpublished data show that pigeons can live in perfect health for a period of at least four months on an exclusive diet of either the whole corn or wheat kernel.

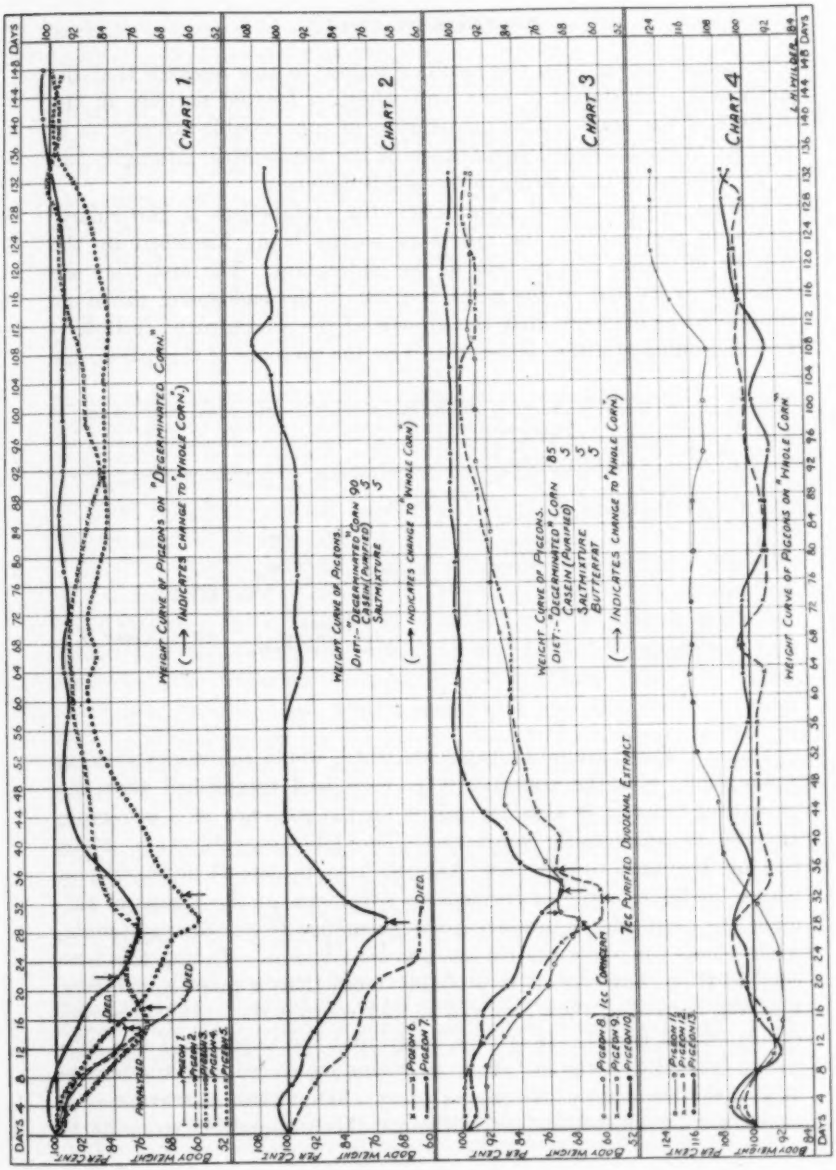


CHART 1
WEIGHT CURVE OF PIGEONS ON "DEGERMINATED CORN"
(→ INDICATES CHANGE TO "WHOLE CORN")
CHART 2
WEIGHT CURVE OF PIGEONS.
DIET:- "DEGERMINATED" CORN 90
CASEIN (PURIFIED) 5
SALTMIXTURE
(→ INDICATES CHANGE TO "WHOLE CORN")
CHART 3
WEIGHT CURVE OF PIGEONS.
DIET:- "DEGERMINATED" CORN 85
CASEIN (PURIFIED) 5
SALTMIXTURE 5
BUTTERFAT 5
(→ INDICATES CHANGE TO "WHOLE CORN")
1 CC PURIFIED ORGANIC EXTRACT
CHART 4
WEIGHT CURVE OF PIGEONS ON "WHOLE CORN"
DAYS
BODY WEIGHT
PER CENT
Pigeon 1
Pigeon 2
Pigeon 3
Pigeon 4
Pigeon 5
Pigeon 6
Pigeon 7
Pigeon 8
Pigeon 9
Pigeon 10
Pigeon 11
Pigeon 12
Pigeon 13
DIED
PARALYSIS
1 CC CONCENTRATE

Charts 1–4

COMMENTS ON RESULTS

It would appear from the results obtained in this research that the antineuritic substance of corn and wheat is located entirely in the embryo of the grain. This discovery is of considerable interest: *a*, in relation to the biological significance of the antineuritic vitamine, and *b*, on account of its bearing on practical dietetics.

During the process of germination the metabolism is particularly active in the embryo, the necessary energy being furnished by the deposit of starch and protein in the endosperm. The fact that the vitamine is located in the germ and not in the endosperm might be considered as evidence that the growth of the young plant (cell division and differentiation) is dependent on the presence of this particular

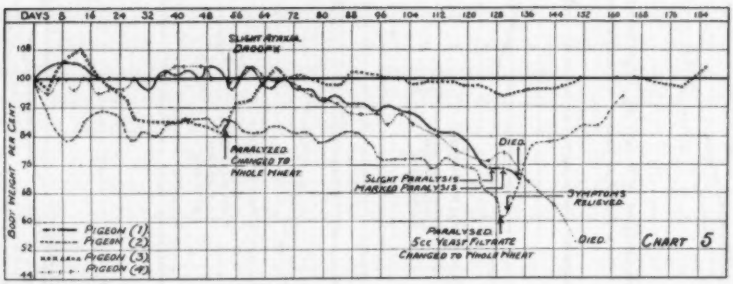


Chart 5

vitamine. There are no known facts which would contradict such an assumption. On the contrary it is very well established that the growth of animals is also dependent on the presence of a minimum amount of antineuritic substance. The following objection might possibly be raised to this view: Animals have to depend ultimately on plants for their supply of antineuritic substance, as the organs of animals are incapable of producing the substance from other materials, while *plants can synthetize the antineuritic vitamine.* Taking this into consideration, however, it is probably more than a mere coincidence that the corn and wheat embryo should contain the entire vitamine supply of the kernel. It is very likely that the presence of this substance is needed in the metabolic processes associated with the formation of the elements of the young plant. What these particular processes are is difficult to say, although the antineuritic vitamine is

perhaps concerned in some way or another with the oxidative functions (see Dutcher and Collatz, 16).

That the antineuritic substance has some relation to metabolism is indicated by various other observations. One of these is the distribution of the substance in the organs of the animal body. From the work of Cooper (7), Osborne and Mendel (8) and others, it would appear that organs with a high rate of metabolism are also rich in antineuritic vitamine. The liver and the heart muscle, for instance, are very rich in vitamine and are also known to be places of especially high metabolic activity. Skeletal muscle, pancreas, kidney, brain, etc., come next in line. It is, furthermore, significant that the first symptoms in so-called "wet" beriberi point to the heart as being primarily affected by the vitamine deficiency of the diet. It is also striking that egg yolk and beans should be rich in vitamine; whereas egg white, the green parts of plants[1] and fruits (such as oranges) are relatively poor in this substance. The yeast cell, with its extraordinarily active metabolism, is also extremely rich in vitamine.

These considerations are summarized in table 1 compiled from the observations of various authors.

To return to the practical aspects of the present research, it might be well to call attention to the bearing of these results on the question of the nutritive value of bread. It is evident that as long as it was assumed that the antineuritic vitamine was located principally in the aleurone cells, it was not so objectionable to remove the germ during the milling process. Our present knowledge, however, would indicate, that by excluding the germ from the flour and cornmeal we deprive these foods of some of their most valuable dietary properties.[2] It should be possible so to alter the milling process as to allow the germ of the cereal to be incorporated in the finished product. A flour or cornmeal containing the germ would, of course, yield bread of a slightly darker color. On the other hand, it is taken for granted that flour or corn meal containing the embryo could not be stored for as long a time as the ordinary highly-milled products, without undergoing some

[1] Since this article went to press a paper by Osborne and Mendel (J. Biol. Chem. 1919, xxxvii, 187) appeared, in which these authors state that dried spinach leaves contain about one-fourth as much antineuritic vitamine as brewer's yeast. The green parts of plants are evidently not as poor in this vitamine as has been assumed hitherto. This observation substantiates our conception of the relation between metabolic activity and antineuritic vitamine, as the metabolism of the leaves of vegetables is certainly very active during the growth of the plant.

[2] The germ also contains a considerable amount of fat soluble vitamine.

deterioration. This is a considerable disadvantage, but could be partly overcome by proper harvesting and storage.

A considerable number of investigators have conceded the higher nutritive value of war bread made from "low extraction" flour (containing the germ and some of the bran). The main objection to this flour is attributed to its excessive cellulose content, which may lead to intestinal disturbances in susceptible individuals. We believe that a product which does not contain any bran but does include the germ, would not possess these objectionable features, would at the same time be more nutritious and would reduce greatly the possibility of vitamine deficiency in the modern mixed diet. Increasing the vitamine supply

TABLE 1

FOOD STUFF	DAILY RATION REQUIRED TO PREVENT POLYNEURITIS (IN TERMS OF DRY WEIGHT)	AUTHORS
	grams	
Yeast (pressed)	0.5	Cooper
Ox liver	0.9	Cooper
Wheat germ	1.3	Chick and Hume
Egg yolk	1.5	Cooper
Ox heart	2.0	Cooper
Fish (hake)	More than 2.0	Cooper
Sheep brain	2.5	Cooper
Lentils	3.0	Cooper
Barley	4.5	Cooper
Beef	5.0	Cooper
Cheese	More than 5.6	Chick and Hume
Cow's milk	More than 3.5	Chick and Hume

in our diet should be our aim in order to insure a sufficient margin of safety, and increasing the vitamine content in our daily bread would go a long way toward accomplishing this end.

SUMMARY

1. The germ or embryo of the wheat and corn kernel contains all of the antineuritic vitamine of these cereals.

2. Wheat flour or corn meal containing the germ is, therefore, more nutritious than the corresponding highly-milled products.

3. Consideration of the distribution of the antineuritic substance in the wheat and corn kernel and in the animal body suggests that this

accessory food is necessary for the metabolism of the growing plant as well as the animal body. It appears that cells with an especially active metabolism are also rich in antineuritic vitamine.

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PRESSURE CHANGES IN THE CEREBRO-SPINAL FLUID FOLLOWING INTRAVENOUS INJECTION OF SOLUTIONS OF VARIOUS CONCENTRATIONS

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In an attempt to determine whether, following intravenous injection of hypertonic solutions of sodium chloride, an increased amount of this salt could be detected in the cerebro-spinal fluid, it was noted that within a short time after the intravenous injection, cerebro-spinal fluid could not be obtained when the subarachnoid space was entered. This observation led at once to the attachment of a manometer to the puncture needle for the purpose of making continuous readings of the cerebro-spinal fluid pressure following the intravenous injection of hypertonic solutions of sodium chloride. It was soon found that the pressure of the cerebro-spinal fluid could be altered very rapidly and very definitely by intravenous injections of solutions of various concentrations.

METHOD

Cats were used entirely in these experiments and the subarachnoid space was entered by puncture with a needle through the occipito-atlantoid ligament. All animals were placed, lying on their sides, on a level board and kept thus throughout the pressure observations. As soon as a puncture was made a glass manometer was connected with the puncture needle by means of a metal elbow. For observations where previous experience made it certain that the pressure would remain above zero, a straight manometer was used, but for observations involving pressures below zero a U-shaped manometer, filled to zero with Ringer's solution, was connected with the puncture needle. The manometer was held in the vertical position throughout the exper-

iment by a burette clamp. As soon as the manometer was attached, direct readings were begun and continued every half-minute for eighty or more minutes. Each reading was recorded as made and curves plotted at the end of each experiment. Our experience has shown that any manipulation of the puncture needle and of the manometer should be avoided if possible. After a good entry of the subarachnoid space uniform conditions are thus assured throughout the period of the experimentation. Sixteen to eighteen gauge puncture needles and manometers with a bore of 1 to 1.5 mm. were used in these observations.

Ether was chosen as the anesthetic largely because experience showed that the concentration of the anesthetic had to be regulated in accordance with the solution injected. It was necessary in many cases to diminish the amount administered for some minutes to avoid death of the animal, particularly when the initial injection was given too rapidly. Although with a non-volatile anesthetic a more even narcosis might be assumed, the disadvantages of this method were found to outweigh its advantages. In the early experiments ether was given by cone but in later observations a very even and satisfactory anesthesia was carried out by the well-known method of intratracheal insufflation. In many of the routine experiments a constant adjustment of the air and ether volume by this method was maintained throughout.

Variation in the temperature of the animals during these long observations under ether was found to affect the general condition of the animal and consequently the pressure of the cerebro-spinal fluid, so that an attempt was made to maintain the body temperature of the animals during the experiments by means of suitably adjusted electric lamps.

Injections of the solutions of different concentrations were all made intravenously. In the early experiments the injections were made with a syringe but owing to the difficulty of control the later injections were all given from a burette connected by rubber tubing with a venous cannula.

NORMAL PRESSURES

A number of readings of the normal pressure of the cerebro-spinal fluid were made in cats under ether anesthesia. At first these readings were isolated observations and as soon as the fluid in the manometer reached a fairly constant level, the record was made. Such observations were made on twenty cats, under routine experimental conditions

and an average pressure of 119 mm. of cerebro-spinal fluid was obtained. The maximal recorded pressure was 155 mm. and the minimal 90 mm.

But these observations were all made on animals which were immediately thereafter used for other purposes. The method of recording the pressure was imperfect in that a considerable dislocation of the cerebro-spinal fluid occurred; this dislocated fluid was required to fill the manometer to the initial level. Likewise the ether was administered by cone, a method which gave varying concentrations of the anesthetic. It was felt that the true pressure of the fluid cou'd only be obtained after sufficient time had elapsed to permit any compensation for the dislocated fluid. In addition, in connection of the manometer to the needle, some fluid was very frequently lost; this loss gave a lower initial reading and necessitated further replacement. Hence experiments were undertaken, with the anesthetic administered by the intratracheal method, extending over a sufficient period of time to permit all physiological compensation for displacement and loss of fluid. For the most part such compensation was accomplished within the first fifteen minutes, as shown by the subsequent records.

In our observations the excursions of the column of cerebro-spinal fluid in the manometer, due to inspiration and expiration, were influenced by the method of anesthesia. With the administration of ether through the intratracheal tube the character and extent of the respiratory movements are altered. With an intratracheal tube of the proper size, sufficient space is left in the trachea and larynx to allow a free return to the exterior. Asphyxia seems to be wholly avoided by the proper regulation of the intratracheal blast. The respiratory movements may be controlled by the volume of the blast; in these experiments it was planned to maintain sufficient movement in inspiration and expiration to give to the column of fluid in the manometer connected with the subarachnoid space, well-marked excursions. Under these conditions these excursions varied from 1 mm. to 6 mm., usually being about 4 mm. in amplitude. These excursions were measured from the low point reached in inspiration to the high point reached in expiration. Pressure on the thorax in our experiments caused a rise of several centimeters in the fluid column in the manometer. Along with the respiratory excursions just noted, there also occur in the fluid column, pulsations due to the heart-beat. In our experiments these pulsations varied from 0.5 mm. to 2 mm. under certain conditions. The usual amplitude of these cardiac pulsations was 1 mm.

Under experimental conditions assuring constant anesthesia and constant body temperature, a number of continuous readings of the pressure of the cerebro-spinal fluid were made over a period of eighty minutes. Figure 1 is a composite constructed from seven such observations and shows the maintenance of a very uniform pressure throughout, though with a slight rise in the readings during the first fifty minutes.

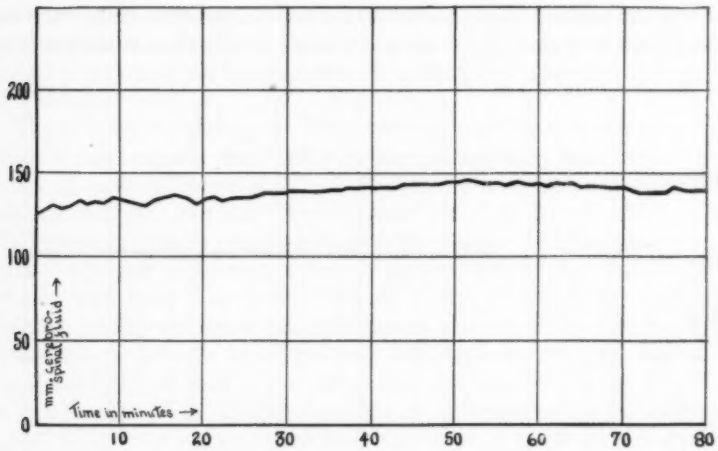

Fig. 1. Composite curve showing normal pressure of cerebro-spinal fluid. Composite made from seven individual curves.

Figure 2 shows a curve of normal pressure of a single cat. It will be noted that in the first five minutes of the observation there is a considerable rise in the pressure. This is quite characteristic in many of our observations and may be accounted for by two factors. First, following the release of the cerebro-spinal fluid by the puncture, dislocation of some of the fluid to fill the manometer was inevitable; second, in the connection of the manometer with the puncture needle the loss of at least one drop of cerebro-spinal fluid occurred in this observation. This initial rise then probably represents, partially at least, the restoration of the fluid displaced and lost in the connection of the manometer. The rise in the curve is unusually abrupt and the compensation seems complete as indicated by the maintenance for the remainder of the experiment (70 minutes) of a uniform pressure. Dur-

ing this observation, when the pressure readings were constantly at the same level, the respiratory excursions in the column of fluid in the manometer were 3 mm. and the arterial variations 0.5 to 2 mm. The uniformity of the pressure readings indicates that under the experimental conditions prevailing, reliable data were obtained.

In figure 1, the composite record of seven observations, it is indicated that the pressure of the cerebro-spinal fluid in the etherized cat was from 135 to 145 mm., with the rise occurring largely in the early part of the curve. Comparable values for the pressure of the cerebro-spinal fluid were obtained from many other cats, both as initial readings

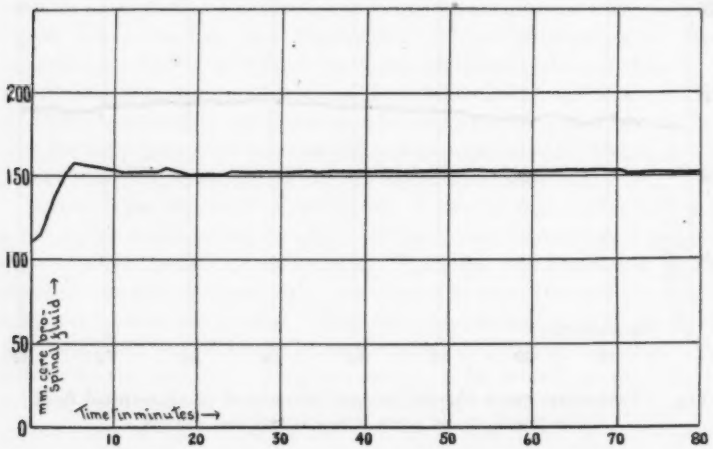

Fig. 2. Cat no. 1319. Normal pressure of cerebro-spinal fluid

and as values of the pressure after the completion of the initial rise. In this series of sixty-five cats, which includes the twenty already mentioned, the initial pressure averaged 119 mm. of cerebro-spinal fluid; the subsequent pressure (after early compensation had occurred), as based on twenty-one animals, averaged 129 mm. of cerebro-spinal fluid. The interesting relationship of the lower value of the initial reading to the later more constant pressure is thereby indicated.

Analysis of the initial pressures of the cerebro-spinal fluid in these sixty-five cases shows that in only eight cats was the reading less than 100 mm. In several of these a loss of three or more drops of fluid, in connecting the manometer, was noted; one animal was obviously in poor

physical condition at the time of experimentation. Others of these eight cats had been subjected to previous experimental procedure in the laboratory. Thirty-six of the sixty-five cats gave initial readings between 100 and 120 mm. with an average of 110 mm., while seven cats fell between 120 and 140 mm. with an average of 131 mm. Fourteen cats showed an initial pressure of over 140 mm. with an average of 153 mm. Five of the fourteen had been previously used for experiments leading to the possible production of a chronic meningitis. In none of these fourteen was the fluid loss in the connection of the manometer more than one drop. Further, the cats which had not been subjected to previous experimentation gave the lower values in this series of fourteen.

The increased elaboration of the cerebro-spinal fluid under the influence of the volatile anesthetics (ether, chloroform) has been noted many times since Cappelletti (1) first described this phenomenon. Pettit and Girard (2), and later Dandy and Blackfan (3) and Dixon and Halliburton (4) have emphasized its importance. All the observations have dealt with an increased production of fluid as determined by the rate of flow from a needle or cannula in the subarachnoid space, the pressure of the cerebro-spinal fluid being determined by the capillary resistance to flow in the cannula. Although no attempt has been made in these experiments to study extensively the effects of variations in the concentration of the anesthetic on the pressure of the cerebro-spinal fluid, some data relating to the pressure of the fluid under these conditions have been obtained.

In our earlier observations, where ether was given by cone, it soon became apparent that variations in the concentration of the ether were sufficient to cause changes of appreciable magnitude in the pressure of the cerebro-spinal fluid as read in the manometer. Analysis of such experiments where there were changes in the pressure of the cerebro-spinal fluid due to variations in the ether supply, shows that the pressure will vary from 5 to 20 mm. with slight changes in the anesthetic during its routine administration by cone. Other, greater changes in the pressure have occurred in animals which were brought rather quickly from very light to full anesthesia or vice versa. Associated with the possible increase in the production of cerebro-spinal fluid due to the ether are other equally significant vascular readjustments; the combination of the two factors within the cranium determines the resulting pressure change in the spinal fluid. Similarly, exhaustion of the secreting cells of the choroid plexus by the anesthesia may present

an additional element which in the latter parts of the observation causes further alterations in the fluid pressure.

Such considerations lead to the view that the pressures recorded as normal in foregoing paragraphs are only relatively and not absolutely accurate. For the values are influenced by the displacement of fluid in the manometer, by loss of fluid in connecting the apparatus and by the anesthesia. The alterations in pressure referable to the anesthetic and associated asphyxia have been controlled throughout so that these factors are constant in all of our observations. Partial or complete compensation for dislocation or loss of fluid is quite uniform and occurs largely during the first few minutes of experimentation. Such relatively constant records of the pressure of the spinal fluid give a reasonable basis upon which further experimental data may be interpreted.

RINGER'S SOLUTION

Following the above observations in which the normal pressure has been plotted over periods of eighty minutes, the effects of various quantities of Ringer's solution, injected intravenously, were considered. The formula for the Ringer's solution used is as follows: NaCl, 0.9 per cent; KCl, 0.042 per cent; $CaCl_2$, 0.025 per cent. In the experiments in which a relatively small amount of the solution (12 to 20 cc.) was injected, the curve showing the pressure of the cerebro-spinal fluid rises during the injection but falls on its completion to the level prevailing at the beginning of the experiment. This pressure is then maintained during the remainder of the observation. Such curves simulate very closely the normal except for the temporary rise during the injection. This initial rise is most marked when the intravenous injection is given very rapidly and with considerable pressure. It seemed apparent then that after the injection of small amounts of Ringer's solution physiological readjustment of the volume of the blood took place very rapidly so that the pressure of the cerebro-spinal fluid was not essentially affected.

Other experiments were performed in which a large quantity (100 cc.) of Ringer's solution was introduced intravenously in order to furnish control for observations (to be described later) in which 100 cc. of water were injected, and to determine if possible the effect on the pressure of the cerebro-spinal fluid of the intravenous introduction of this enormous volume of a solution isotonic with the blood. Figure 3 shows a record of the pressure of the cerebro-spinal fluid in such an

experiment. In this case the injection was made from a burette connected with a cannula in a fore-leg vein and lasted for twenty-six minutes, being given then at a rate of 3.85 cc. per minute. During the injection of the first 33 cc. the rate was rapid (6.6 cc. per minute); and for the next 17 cc. the rate was 4.25 cc. per minute. Reference to figure 3, in which the rapidity of the injection is indicated on the base line showing the period of injection, reveals the fact that when the rate of introduction of the fluid was high, at the beginning of the injection, the pressure of the cerebro-spinal fluid rose more abruptly and more rapidly than when the rate was decreased. The mechanical effect of a difference in the rate of intravenous injection, on the pressure of the cerebro-spinal fluid, is thus indicated. A further matter of interest brought out by our observation during the injection period in this

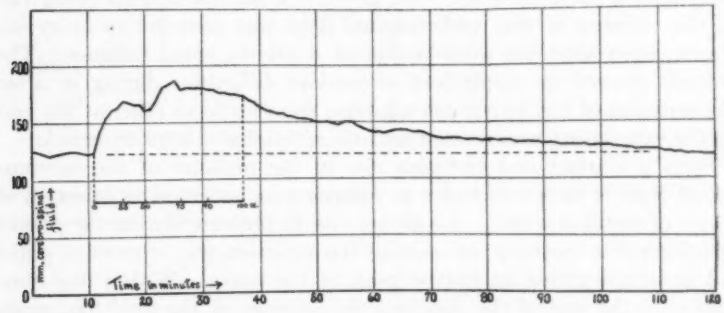

Fig. 3. Cat no. 1543. Pressure cerebro-spinal fluid with intravenous injection of 100 cc. of Ringer's solution.

experiment, is that the impact of the injection seems to have an effect on the pressure of the cerebro-spinal fluid which is independent of the effect produced by rapid injection. This has been noted in many of our experiments. During the latter part of the period of injection the rate was low and it can be seen from figure 3 that the pressure of the cerebro-spinal fluid had already begun to fall. This decrease in pressure continued gradually and uniformly during the remainder of the observation, the fall gradually becoming less rapid. Within thirty minutes after the completion of the injection the pressure had dropped to 138 mm., a point probably well within the normal pressure variation in this animal. During the last thirty minutes of the experiment the pressure, which was falling very gradually, was practically the same as that noted before the injection of the Ringer's solution.

It is apparent from the foregoing observation that the intravenous injection of a relatively large volume of fluid isotonic with the blood, although it increases the pressure of the cerebro-spinal fluid during the period of injection, does not bring about any material and lasting change in this pressure. The physiological adjustment to such a large volume is, however, a process requiring more time than is necessitated by the intravenous injection of smaller volumes. No ill-effects of this procedure are apparent in animals allowed to recover from the anesthetic.

HYPOTONIC SOLUTIONS

With the establishment of a curve of normal pressure of the cerebro-spinal fluid under the experimental conditions adopted, intravenous injections of distilled water were given. A prompt and enduring rise in the pressure of the cerebro-spinal fluid was recorded in every instance, even after the introduction of relatively small volumes. The animals showed no respiratory or cardiac difficulties during or after the injection of this hypotonic solution; recovery from ether at the end of the experiment was prompt and no after-effects were observed.

Such a marked and enduring rise in the pressure of the cerebro-spinal fluid is shown in figure 4, following an intravenous injection of 20 cc. of distilled water. An abrupt rise in pressure during the period of injection is recorded; as soon as the injection was stopped a slight fall occurred, giving an obtuse peak in the curve. Within five minutes after the end of the injection the pressure of the fluid had again started to rise and this upward tendency was maintained throughout the period of observation.

An increase in the pressure of the cerebro-spinal fluid is the typical response to the intravenous injection of a hypotonic solution (distilled water). The initial peak in the curve during the injection seems to be partially at least a mechanical effect due to the rapid introduction of fluid into the venous system. It may be lessened or avoided by the very slow injection of the foreign solution, indicating that the resultant persisting change in the pressure of the cerebro-spinal fluid is not an immediate phenomenon but requires a varying interval of time for its fulfilment. This interpretation is supported by the curves in figures 4 and 5; in both of these animals, especially in the one receiving the smaller dose, the effect of the hypotonic solution is continued and is at its maximum at the end of the observation. That in the intravenous injection of even a large amount of distilled water, the initial

peak in the pressure curve may be avoided by giving the injection uniformly and gradually over a considerable period of time (26 minutes), is shown by figure 5. This curve may be compared

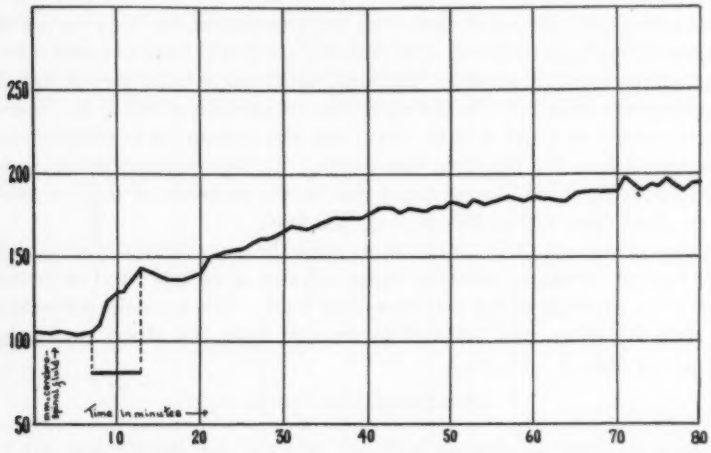

Fig. 4. Cat no. 1303. Pressure cerebro-spinal fluid with intravenous injection of 20 cc. sterile distilled water.

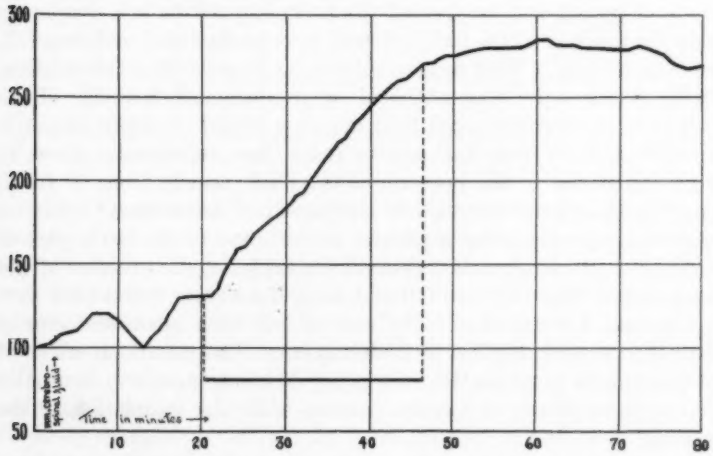

Fig. 5. Cat no. 1498. Pressure cerebro-spinal fluid with intravenous injection of 100 cc. sterile distilled water.

directly with figure 3, which represents the pressure of the cerebro-spinal fluid after the intravenous injection of a similar amount of Ringer's solution. In figure 5 the rise in pressure begins abruptly, is maintained throughout the period of introduction of fluid and continues even after its completion. In this experiment the long period of injection may have sufficed to include within it the time necessary for the readjustment of pressure relations, so that any fall after the initial injection was avoided. In the early part of the curve, before the injection of water, is a fall due to ether; but the pressure was constant at 130 mm. before the injection was begun. In this experiment, as seen from figure 5, there is recorded a rise in the pressure of the cerebro-spinal fluid from 130 to 285 mm. of this fluid.

These observations show definitely that the intravenous injection of a hypotonic solution (distilled water) causes a prompt and enduring rise in the pressure of the cerebro-spinal fluid. The animals, subjected to such injections and allowed to recover from the ether, promptly become normal.

HYPERTONIC SOLUTIONS

Many experiments dealing with the effect of the intravenous injection of concentrated solutions of the common sodium salts (chloride, bicarbonate and sulphate) upon the pressure of the cerebro-spinal fluid have been carried on. In general, this procedure results in a temporary rise in the pressure of the fluid followed by a marked and enduring fall.

Sodium chloride. With sodium chloride in 30 per cent concentration, striking results have been obtained, with continuous readings of the pressure of the cerebro-spinal fluid during a period of eighty minutes. All curves plotted from data arising from these experiments show an abrupt initial rise in the pressure of the fluid, usually from 50 to 75 mm. This continues throughout the period of intravenous injection though it frequently forms a plateau in the curve in the latter part of the process. As soon as the injection is completed, the pressure of the cerebro-spinal fluid starts to fall and a rapid lowering is recorded during the next few minutes. The rate of fall then decreases shortly before the pressure reaches its lowest point. The maximum effect of the intravenous injection, i.e., the point of lowest pressure, is usually noted in from fifteen to twenty minutes after the completion of the injection.

Figure 6 shows the effect of a single intravenous injection of 12 cc. of a 30 per cent solution of sodium chloride upon the pressure of the

cerebro-spinal fluid. An initial rise of 80 mm. during the period of injection is recorded; following this a rapid and extreme lowering of the pressure occurred, the rate of fall decreasing only when the pressure went to below zero. The minimal pressure of the cerebro-spinal fluid recorded was minus 22 mm., a point reached eighteen minutes after the completion of the injection. The pressure then remained below zero for a period of twenty-six minutes. Subsequently it rose slowly to a pressure of 40 mm. above zero, which was reached at the end of the observation.

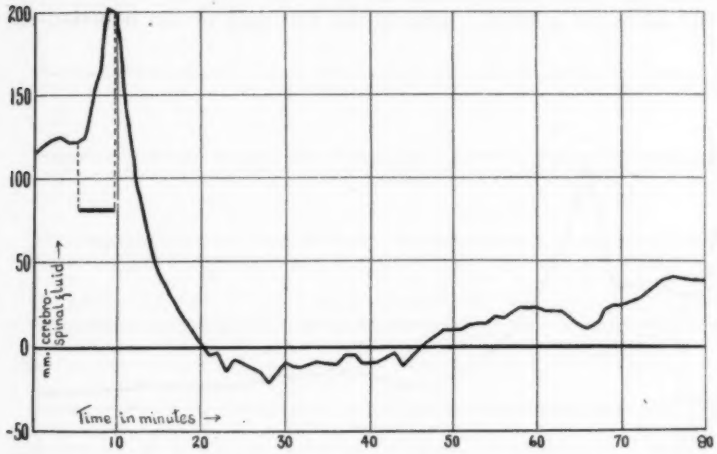

Fig. 6. Cat no. 1271. Pressure cerebro-spinal fluid with intravenous injection of 12 cc. 30 per cent sodium chloride.

It must not be assumed that the intravenous injection of concentrated solutions of sodium chloride inevitably brings about a lowering of the pressure of cerebro-spinal fluid to below zero. With various intravenous doses of this salt, roughly half of our experiments gave readings below zero. The maximum effect was obtained in one cat by repeated intravenous injections (total 22 cc.) of 30 per cent sodium chloride, when the pressure of the cerebro-spinal fluid was recorded as minus 55 mm.

In many other observations, the result of such injections of sodium chloride was a marked lowering of the pressure of the cerebro-spinal fluid (50 mm.) and the maintenance of this lowered pressure through-

out the period of record. This seems to be the phenomenon of chief physiological importance—a reduction of the pressure to a lower level. A curve showing the result of an experiment of this nature is given in figure 7. In this case the pressure dropped abruptly for a period of ten minutes after the intravenous injection; this lowered pressure was maintained with minimal change for at least forty minutes.

Sodium bicarbonate. Of the six cats given intravenous injections of saturated solutions of sodium bicarbonate (about 18 per cent), only one showed a lowering of the pressure of the cerebro-spinal fluid to below zero. This animal was given an intravenous injection of 10 cc. of the saturated solution. During the first part of the injection the

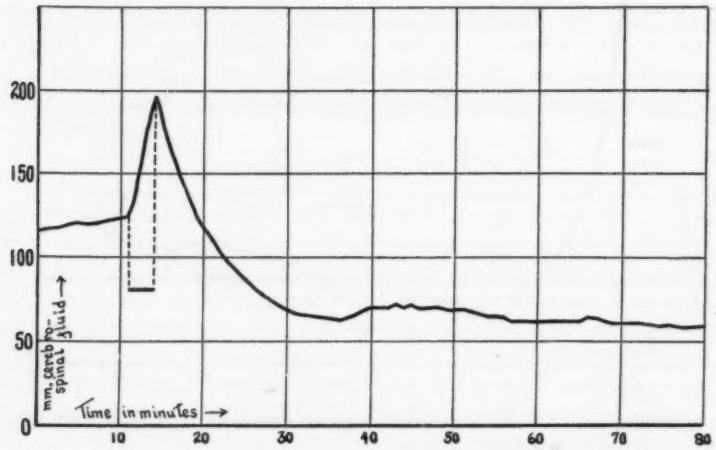

Fig. 7. Cat no. 1395. Pressure cerebro-spinal fluid with intravenous injection of 10 cc. 30 per cent sodium chloride.

pressure of the cerebro-spinal fluid rose 40 mm. but before the completion of the injection a rapid fall had begun. This break in the initial rise before the completion of injection is recorded in most of the curves with this salt. The marked fall continued abruptly in this case to 12 mm. below zero, a point reached in sixteen minutes after the end of the injection. This pressure remained at zero or below for a period of forty minutes and then slowly started upwards. This curve is reproduced in figure 8.

The more common response in the pressure of the cerebro-spinal fluid to intravenous injection of sodium bicarbonate is merely a lowering of 40 to 70 mm. The curves show typical peaks with breaks before

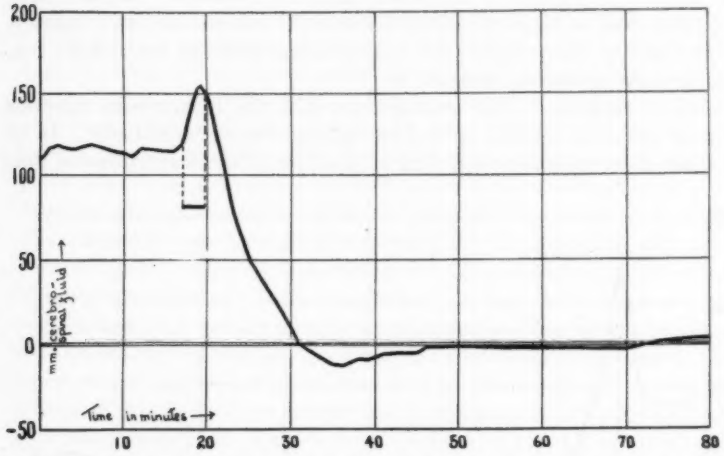

Fig. 8. Cat no. 1364. Pressure cerebro-spinal fluid with intravenous injection of 10 cc. saturated solution of sodium bicarbonate.

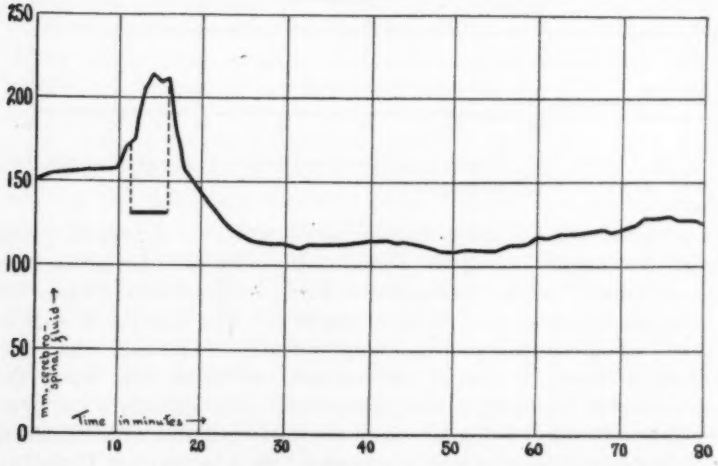

Fig. 9. Cat no. 1365. Pressure cerebro-spinal fluid with intravenous injection of 18 cc. saturated solution of sodium bicarbonate.

the completion of the injections. One of these typical curves with a lowering of the pressure of the cerebro-spinal fluid of approximately 40 mm. is given in figure 9. The injection in this case was 18 cc. of the saturated solution of the bicarbonate; the difference in reaction to a smaller dose in another animal is shown by comparison with figure 8. At the end of the period of observation some recovery toward the normal pressure is shown in figure 9.

Sodium sulphate. Four experiments with the intravenous injection of a 30 per cent solution of sodium sulphate were carried out. In all of these observations a reduction of pressure of the cerebro-spinal fluid

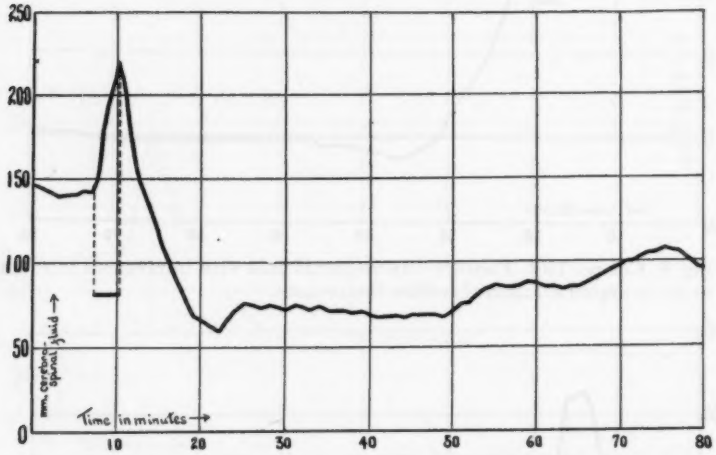

Fig. 10. Cat no. 1356. Pressure cerebro-spinal fluid with intravenous injection of 10 cc. 30 per cent sodium sulphate.

was recorded, the fall being usually 50 to 80 mm. A typical initial rise in the pressure during the period of injection with formation of a peak is shown by all the curves, one of which is reproduced in figure 10. In this case an injection of 10 cc. of the 30 per cent solution of sodium sulphate was given; this gave an abrupt initial rise of 80 mm. which was promptly followed by a rapid fall to a point about 90 mm. below the pressure at the beginning of the experiment. Subsequently a low pressure (about 70 mm.) was maintained for thirty minutes with a gradual rise but incomplete recovery in pressure. Such a curve is typical of the series; none have shown pressures below zero.

Glucose. The observations in the foregoing paragraphs of this section have all been concerned with the lowering of the pressure of the cerebro-spinal fluid by the intravenous injection of concentrated solutions of electrolytes (common sodium salts). In an attempt to determine whether all crystalloids possess this power of bringing about a lowering of the pressure of the cerebro-spinal fluid, four experiments were undertaken in which intravenous injections of a concentrated solution of glucose were given. Large amounts of a 30 per cent solution were given and typical responses were obtained. The curves all show a marked abrupt initial rise followed quickly by a fall to a level lower than that prevailing at the beginning of the experiment. This lowering was not as marked as with the electrolytes but in two of the experiments the resultant level was 60 mm. below the initial pressure.

Toxicity and dosage of the sodium salts. It was early noticed that the intravenous injection of the concentrated solutions of sodium chloride was followed by severe respiratory and cardiac disturbances. It was often necessary to alter the anesthetic to avoid losing the animal. The ether was in these cases reduced and artificial respiration used, the intratracheal method of anesthesia making possible a nearly ideal artificial respiration. This toxicity of the sodium chloride was apparently not dependent upon the absolute amount injected but was frequently observed in the earliest intervals of the injection when only 1 to 2 cc. had been introduced. As soon as this initial toxicity was passed, further injection up to certain limits could be made with comparative safety in the same animal. Such early disturbances seem best explained on the basis of an alteration in the balance of the mineral salts in the blood.

In addition to the initial toxicity of the sodium chloride, another factor of importance in these experiments was variation in the tolerance of the individual animal. Some animals, showing no apparent disturbance on receiving relatively large intravenous injections, have given marked reactions in the pressure of the cerebro-spinal fluid following such injections. Other animals under similar conditions have exhibited but little reaction as indicated by a lowering of the pressure of the cerebro-spinal fluid. In still other animals where the toxic effects of the salt were marked, the lowering of the pressure of the cerebro-spinal fluid was definite and considerable. Thus an individual tolerance and reaction for each particular animal seems indicated, a tolerance and reaction which we have not been able to predict in our experiments.

Quite similar to these experiences with intravenous injections of sodium chloride were our findings with the bicarbonate and sulphate. It has already been pointed out that in one case the injection of 10 cc. of the saturated bicarbonate gave a far greater fall in the pressure of the cerebro-spinal fluid than an injection of 18 cc. of the same salt in another animal (cf. figs. 8 and 9). Such individual variations are common also with the sulphate, although the final toxicity of the salt used in our experiments seems much greater. Few animals have shown any disturbance during the injection of the sulphate solution but several have died, apparently from the toxic effect of the salt, before the completion of the observation.

Following the intravenous injection of these concentrated salts, then, the character of the resultant curve, as indicating the extent of the reaction producing the lowering of the pressure of the cerebro-spinal fluid, must be referred partially at least to the individual tolerance and reaction of the animal used in the experiment. The definite and marked lowering of the pressure by the intravenous injection of concentrated solutions of electrolytes is shown in all the curves, but the degree of reaction seems dependent, to some extent at least, on the individual animal. All of these animals, if allowed to recover from the ether, seem slow and quiet for a few hours; within twelve hours they become normal in every way.

DISCUSSION

In a foregoing part of this paper the results of the reading of the pressure of the cerebro-spinal fluid in sixty-five cats have been set forth. The average of the initial readings in these cases was 119 mm. but it was pointed out that if readings were taken a few minutes later, a higher value (129 mm.) was obtained. This higher value, as has already been indicated, is probably to be accounted for by replacement of fluid which follows the dislocation and loss due to the mechanical arrangement of the experiments. It seems reasonable to assume then, that, after sufficient time has elapsed to allow replacement of fluid which occurs after the puncture and connection of the manometer, we are dealing with a pressure of the cerebro-spinal fluid which is truer normal than would be obtained by readings taken immediately after puncture.

There are available in the literature records of observations on the pressure of the cerebro-spinal fluid which have been made on dogs

under various anesthetics and by various methods. So far as is known to us, no observations of this sort have been made on cats. In general, our readings on cats agree fairly well with those recorded for dogs. They are somewhat lower than those given by Key and Retzius (5) (162 to 216 mm. of water in inspiration; 216 to 270 mm. of water in expiration—dogs under ether); higher than Bergmann's first observations (6) (80 mm. of water, narcotized dogs) but agreeing fairly well with his later findings (7) (120 to 160 mm. salt solution, narcotized dogs). They coincide fairly accurately with the records of the work of Falkenheim and Naunyn (8) (100 to 150 mm. of water, dogs curarized), and while the conditions in Leyden's experiments (9) are not comparable with those prevailing in ours, his results (80 to 150 mm. of water, 100 to 120 mm. of water, dogs under morphia) seem well in accord with ours. It is felt that the method of intratracheal anesthesia and the direct readings of the pressures in the manometer as practiced in our experiments give a better index of the normal than was obtained by Dixon and Halliburton using a graphic method and morphine-urethane anesthesia. Dixon and Halliburton (10) give a rough average of 40 to 70 mm. of salt solution as the normal pressure of the cerebro-spinal fluid in the dog, but the extreme variations which they record have not been noted in our observations on control animals. Thus they record as typical variations of the normal pressure of the cerebro-spinal fluid in the dog, the following values read at five-minute intervals: 95 – 25 – 30 – 35 – 55 – 25 – 80 – 65 – 65 – 75 – 70 – 60 – 55 –50 – 80 – 90 mm. of 10 per cent sodium citrate solution. Compared to the curve reproduced in figure 2, in which the respiratory and cardiac excursions were well marked throughout, these variations recorded by Dixon and Halliburton seem extreme.

The logical physiological explanation of the alterations in the pressure of the cerebro-spinal fluid following the injection of hypertonic and hypotonic solutions, seems naturally to be concerned with the experimental change in the osmotic value of the blood. Thus an increase in the concentration of the fluids injected resulted in a fall in the pressure of the cerebro-spinal fluid while the injection of a hypotonic solution gave an enduring rise. Data regarding the relative or absolute osmotic values of the blood have not been obtained. It would appear that the alteration in the salt content of the blood produced experimentally can only be compensated by fluid readjustments within the tissues, for renal function is more or less impaired by the anesthesia. The individual reaction of the animal noted above may

therefore be referred to variation in the fluid interchange between blood and tissue, so that alterations in the pressure of the cerebro-spinal fluid following intravenous injections of hypertonic and hypotonic solutions seem dependent on the osmotic readjustments within the tissues.

A number of observations have been made on the pressure of the cerebro-spinal fluid with simultaneous record of the arterial blood pressure, heart-rate and venous pressures. Further experiments of this kind are under way and will form the basis of a future report.

SUMMARY

1. The pressure of the cerebro-spinal fluid in the etherized cats has been found to average 119 mm. of this fluid, if read immediately after the connection of the manometer with a needle in the subarachnoid space; but this pressure, if read some minutes later, is somewhat higher, giving an average of 129 mm., this rise being due, partially at least, to the replacement of cerebro-spinal fluid displaced in the manometer or lost during its connection.

2. Intravenous injections of Ringer's solution cause no lasting change in the pressure of the cerebro-spinal fluid.

3. Intravenous injections of hypotonic solutions (distilled water) are followed by a marked and sustained rise in the pressure of the cerebro-spinal fluid.

4. Intravenous injections of hypertonic solutions (concentrated sodium chloride, sodium bicarbonate, sodium sulphate and glucose) cause initial rise in the pressure of the cerebro-spinal fluid followed immediately by marked fall in this pressure, often to below zero.

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EXPERIMENTAL ALTERATION OF BRAIN BULK

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In the early stages of an investigation of the factors underlying the swelling (edema) of the brain in acute infections or injuries, attention was directed to the possible relationship between the volume of the brain and the alteration in the pressure of the cerebro-spinal fluid, following intravenous injections of solutions of various concentrations (1). For in the study of cerebral edema, but little progress has in the past been made on account of the difficulty of experimental approach. This condition remains today one of the great problems in pathology of the central nervous system.

The marked changes in the pressure of the cerebro-spinal fluid, reported in the foregoing paper, were quickly found to have a definite relation to the resultant volume of the brain. Thus, following intravenous injections of strongly hypertonic solutions which markedly lowered the pressure of the cerebro-spinal fluid, definite shrinking of the brain occurred. And conversely the brain bulk was appreciably increased by the intravenous injection of hypotonic solutions, which raised the pressure of the cerebro-spinal fluid. Such changes in the size of the brain are rapidly and uniformly brought about, giving definite information as to one phase of the physiological regulation of the volume of this organ.

METHODS

Cats were used entirely in this work. Intravenous injections of the various solutions were given with a syringe or with a burette connected directly with a fore-leg vein. For the hypertonic solutions, 30 per cent sodium chloride or saturated sodium bicarbonate in distilled water were given, as previous work had demonstrated their efficacy in lowering the

pressure of the cerebro-spinal fluid. Ringer's solution (NaCl, 0.9 per cent; KCl, 0.042 per cent; $CaCl_2$, 0.025 per cent) was injected intravenously to give data regarding the possible alteration in the circulation and in the volume of the brain brought about by the introduction of an increased volume of fluid, while distilled water was used as the hypotonic solution. Cats which had been given the customary intravenous injections of these solutions and then allowed to recover from the anesthetic, were usually a little slow for six hours but became normal and active within twelve hours. For the most part the observations were carried out on cats with unopened skulls, but in two series, subtemporal trephine openings were made, not only to relieve the intracranial tension but also to permit direct observation of the brain.

All animals used in these observations were anesthetized with ether, usually by intratracheal insufflation but in the earlier experiments by cone. The body temperature of the animals was maintained throughout. After the lapse of time necessary for the maximum action of the solution intravenously introduced, the animals were killed by ether. In the routine experiment, 10 per cent formalin was injected, immediately after death, through the aorta at a pressure of not more than 800 mm. of water. When the cranial vessels were well filled, the central nervous system was removed (the skull and vertebral canal being partially opened) and the whole immersed in 10 per cent formalin. In spite of all the care it was possible to exercise, it was soon very evident that by this method of fixation, the form and size relations of the central nervous system prevailing prior to the death of the animal were not being accurately preserved. Brains markedly shrunken during life or at death of the animal approached almost normal proportions after such fixation, and brains markedly herniated at death often subsided perceptibly during preservation. The brains of other animals were fixed by direct immersion in formalin; the results of this method were similar in regard to alteration in volume. The addition of a suitable amount of sodium chloride to the formalin solution did not prevent these changes in brain bulk during preservation and was as unsatisfactory as other solutions.

Although the method of fixation used is inadequate, still it enables one to make general comparisons of the brains after various intravenous injections, even though it does not preserve the volume relations accurately. It is hoped there will be found a means of fixation which will preserve more exactly the form and size relations and at the same time make possible a study of the histology and cytology of these brains with reasonable confidence.

EXPERIMENTS WITH UNOPENED SKULL

The majority of these experiments were carried out on animals without opening of the skull. Some of this series, however, were used for determinations of the pressure of the cerebro-spinal fluid; in these the subarachnoid space was entered by a needle through the occipito-atlantoid ligament for connection with a manometer. In this limited manner the pressure relations within the cranium may be considered to have been altered; other animals of this series were carried through with intact cranial cavities.

Normal. Under this heading it is purposed to discuss the normal bulk of brains as found in cats killed without experimentation, and in those given Ringer's solution in such amounts as to control the volume of the other intravenous injections. The intravenous injections of Ringer's solution in these quantities did not alter the volume or appearance of the brain, so that, as far as our observations go, the brains of these animals are to be classed as normal.

When removed from the skull after routine fixation with formalin, the normal brain surrounded by unopened dura presents in the cat typical appearances. The dura over the convexities is only fairly well filled out and is under no appreciable tension, falling slightly between the adjacent gyri. On looking through the dura, a definite rounding of the convolutions and the fairly well-defined edges of the sulci are apparent. On transverse section of the normal brain (fig. 3), differentiation between gray and white matter is obvious. At the periphery of the section, the dura is seen to fall slightly between adjacent gyri. The surfaces of the gyri present smoothly-rounded curves, dipping into well-defined sulci of appreciable width. The median longitudinal fissure is clear cut and the adjacent gyri definitely separated. A line formed by the arachnoid membrane can be made out, bridging the larger sulci. Quite similar appearances are presented by the brains of animals receiving intravenous injections of Ringer's solution (fig. 4).

Examination of a large series of cats' brains fixed under similar conditions has shown that considerable individual variation exists. In brains of old animals, the gyri appear more rounded and the sulci deeper than in the younger cats. The dura in such older animals seems looser and denser, suggesting the various phenomena of old age exhibited by the brain in man. In very young cats and kittens, there seems to be a tendency toward swelling following formalin fixation. These individual variations, according to age, must be constantly

borne in mind while interpreting the results of the experimental modification of the volume of the brain.

Hypotonic solutions. The intravenous injection of water, which has been found to produce a definite increase in the pressure of the cerebro-spinal fluid, causes also a frank swelling of the substance of the brain. Amounts varying from 20 cc. to 100 cc. were injected intravenously; the degree of the reaction was apparently not dependent upon the absolute quantity of water injected, for the maximum effect observed occurred in a cat receiving only 20 cc. of water intravenously. Figure 1 gives the gross appearance of the formalinized brain of a cat which had been subjected to an intravenous injection of 35 cc. of distilled water, and sacrificed thirty-five minutes after completion of injection. The dura over the cerebral hemispheres is markedly tense as in all others similarly treated. The convolutions appear flattened when viewed through the dura and the sulci are traced with greater difficulty than in the normal. On passing the finger over the dura covering the upper surface of the brain, one receives an impression of marked tenseness of dura and brain, and recognizes the gyri and intervening sulci with difficulty.

On section, such brains (figs. 5 and 7) exhibit the same tenseness of dura previously noted. The normal differentiation between the gray and white matter has been diminished (figs. 3, 5 or 7). The convolutions appear definitely flattened, adjacent gyri being pushed together so as to make the identification of the intervening sulci difficult. This is particularly true of the smaller sulci. The surfaces of the gyri are no longer gently convex but acute angles in the curve are found where the surface dips into the sulci. The superior longitudinal fissure is narrow and the bounding gyri press tightly against the falx. The cut surface of the brain appears definitely turgid and gives the impression of having been subjected to increased tension.

An increase in the volume of the brain following the intravenous injection of water is therefore definite, marked and readily apparent.

Hypertonic solutions. The intravenous injection of strongly hypertonic solutions, which has been found to cause a profound lowering of the pressure of the cerebro-spinal fluid, has been observed to produce also a decrease in the bulk of the brain. This alteration in the volume of the brain has been brought about by intravenous injections of from 8 cc. to 20 cc. of 30 per cent sodium chloride or saturated sodium bicarbonate. As reported elsewhere under the subject of alterations in the cerebro-spinal fluid pressure, a marked individual variation in reaction

to hypertonic solutions has been found to exist. The dose bringing about the maximal cerebral shrinkage varied, but in general the larger doses, approaching the limit of the animal's tolerance, seemed to cause the most marked effect. Some of the cats were given single injections of as much of the hypertonic solution as they seemed able to stand at one administration. Other animals were given a series of 5 cc. doses of 30 per cent sodium chloride at half-hour intervals, until a total of 20 cc. was injected. The animals, except when given the divided doses at half-hour intervals, were kept under ether anesthesia until sacrificed.

The time necessary for the maximum action of the fluid injected could not be determined accurately for the cats with unopened skulls. Assuming however, that the maximum lowering of the pressure of the cerebro-spinal fluid after such an injection coincides with the maximal diminution of brain-volume, it is probable that an interval of fifteen to twenty minutes suffices for the maximum change. Within certain limits, these observations substantiate this assumption, namely that the amount of fall in pressure of the cerebro-spinal fluid is an index of the extent to which the volume of the brain has been reduced.

The brain of a cat after the intravenous injection of a strongly hypertonic solution shows, on routine formalin fixation, a marked decrease in volume. As seen through the dura (fig. 2), which is very loosely applied, the brain seems comparatively small, occupying only a part of the intradural space. The gyri appear markedly rounded and the sulci wide and deep, so that individual convolutions appear throughout their extent. In the medulla oblongata and spinal cord there is also evidence of marked decrease in size. The dura here is very much more loosely applied than in the normal and the markings of the medulla oblongata and spinal cord seem sharp and accentuated. The general impression received from such an uncut brain is that it is quite small in comparison to the dural sac.

When cut transversely, the brain from a cat subjected to this experimental procedure presents an appearance quite different from the normal. The gray and white matter are far more sharply contrasted. Furthermore, the gray matter, particularly that of the thalamus and corpus striatum, appears dark with a brownish tinge, clearly outlining the nuclei from the adjacent white fibers. This phenomenon has been noted quite uniformly in this series. On such a section, the dura is very loosely applied (fig. 6), touching on the dorsal surface only the highest points of the gyri. Each individual gyrus stands out cleanly separated from adjacent gyri by widely opened sulci. The curve

presented by the upper surface of each gyrus is of smaller radius than the normal, and may be followed deeply into each sulcus. The superior longitudinal fissure gapes widely and the falx seems to hang loosely within this space.

Similar shrunken brains have been obtained by the intravenous injection of saturated solutions of sodium bicarbonate. A section of such a brain is shown in figure 8, which presents in general the characteristic features noted above. The decrease in volume is, however, not so striking as in the brain reproduced in figure 6.

It was thought possible that the action of these hypertonic solutions might be enhanced by depriving such animals of all fluid for a sufficient length of time before experimentation to insure the exhaustion of a considerable quantity of the water available in the body. Two series of animals were thus prepared and injected with 30 per cent sodium chloride in 5 cc. doses at one-half hour intervals. While the cats receiving four 5 cc. doses showed the effects of the injection in a very marked way (gross clonus of whole body, mild mania, etc.), the brains failed to show any more, if as much shrinkage as is shown by the brains of animals not denied water. These observations indicate that deprivation of water for twenty-four hours in the cat is not sufficient to alter the fluid-volume of the body tissues available for reaction with hypertonic solutions.

In the unopened skull, then, a definite decrease in the bulk of the brain may be brought about by intravenous injection of strongly hypertonic solutions.

The supply of a foreign solution to subarachnoid space. At the end of a number of the experiments in which an intravenous injection of hypertonic sodium chloride was given, a mixture of sodium ferrocyanide and iron-ammonium citrate was allowed to flow into the subarachnoid space. This was done at a time when the pressure of the cerebrospinal fluid was about zero or falling rapidly. Two or three cubic centimeters were usually so introduced. At the end of the experiment the animal was injected through the aorta with 10 per cent formalin, to which 5 per cent hydrochloric acid had been added, and when the vessels were well filled the central nervous system was quickly removed and immersed in the acid formaldehyde. By this procedure, Prussian blue was precipitated at the points to which the solutions of sodium ferrocyanide and iron-ammonium citrate had penetrated prior to fixation. The Prussian blue in almost every case was found to have passed from the subarachnoid space along the perivasculars into the substance

of the nervous system, reaching the interfibrous spaces in the white matter and the pericellular spaces in the gray. These observations may be interpreted as indicating that the hypertonic solution of sodium chloride, injected intravenously, had caused the dislocation of a considerable quantity of the cerebro-spinal fluid into the nervous system.

EXPERIMENTS WITH THE OPENED SKULL

The experiments dealing with the alteration of brain bulk by intravenous injection of solutions of varying concentrations were, in the earlier part of this investigation, carried out with the unopened cranial cavity, in which a fairly constant fluid volume was necessarily maintained. Even with such limitation to change in the volume of the brain, these solutions of various tonicities caused marked modification in its size. It was therefore thought desirable to carry out similar observations but with opening of the skull to permit expansion or contraction of the brain—changes impossible under the physical conditions imposed by the closed cranium. The rate of reaction to the intravenous injection and the appearance of the brain throughout the experiment could also, under these conditions, be determined by direct observation.

The opening of the skull was accomplished in the subtemporal region. In the etherized animal, through a midline incision, the temporal muscle on one side was freed from its origin, and a trephine opening of 2 cm. made in the skull beneath the muscle. The upper border of this opening came within 3 mm. of the mid-sagittal line of the skull. In another series bilateral subtemporal decompression openings were made. In every case the dura was freely opened by cruciate incisions. Injury to the underlying arachnoid and brain was carefully avoided.

Normal. Control experiments with single and bilateral decompressions were carried out. Following the opening of the skull, the animal was kept under ether until sacrificed at the expiration of about the same length of time as was consumed in the experiments where intravenous injections were given. In these control animals under such conditions the brain lay slightly convex beneath the trephine opening, pulsating freely, and did not change perceptibly in any way during the period of observation. The dural flaps were allowed to lie loosely on the exposed surface of the brain and throughout the experiment the edges of these flaps were separated from 1 to 2 mm. in the center of the trephine opening. At the end of these observations the animals were sacrificed

with ether and the brains immediately preserved in 10 per cent formalin. On section, these brains appear quite normal with evidence of slight dislocation of brain substance toward the site of the trephine openings. Figure 13 shows a section of such a brain which was relieved by a single opening in the skull and dura; figure 16 represents the condition prevailing after a bilateral subtemporal decompression. It may be concluded, then, that under the conditions of experimentation the volume of the brain has been but little changed by the anesthesia and operative procedures employed.

Further control of the observations of brain bulk, following intravenous injection of solutions of various concentrations, is afforded by several experiments in which Ringer's solution was injected intravenously after single or bilateral openings had been made in the skull and dura. The injection of Ringer's solution was from a burette, and the rate of fluid-introduction was regulated to coincide with that used in the injection of similar amounts of hypotonic solutions. During and after the injection of Ringer's solution, in amounts up to 100 cc., the brain lay slightly convex in the trephine openings, pulsating freely, and showed no evidence that it had been affected in volume by the intravenous injection. The appearance of the cortex viewed through the trephine opening was exactly that of the brain of an animal receiving no intravenous injection, but subjected to the other operative procedures. In figure 10 is shown the result (after fixation in formalin) of an experiment in which a single opening was made in the skull and 100 cc. of Ringer's solution injected intravenously. As pointed out previously, we have been unable with our present methods to preserve, by fixation in formaldehyde, the form and size relations prevailing in the brain at the end of experimentation. This figure shows a slightly more marked bulging of the brain in the trephine opening than was present at the end of the experiment. In spite of this slight swelling due to fixation it presents a fairly normal appearance, particularly when compared with more swollen or shrunken brains as shown in figures 9 and 11. In this and in all of our observations in which Ringer's solution was injected, the anesthesia, the operative procedures, the time consumed by the intravenous injection and the interval of time from the end of the injection to the sacrifice of the animal, were similar to those in the experiments in which solutions of various concentration were injected.

It may be concluded, then, that in etherized animals with the skull opened, the intravenous injection of Ringer's solution in amounts up to 100 cc. causes no appreciable change in the volume of the brain. The

protocol of a typical experiment in which there was a bilateral opening of the skull and the intravenous injection of 100 cc. of Ringer's solution, is given below:

No. 1537. Adult male cat. Weight 2500 grams. Control intravenous Ringer.

9.46 a.m. Ether with intratracheal tube.
10.15 a.m. Double subtemporal decompression. Dura opened. Brain lies with normal convexity, pulsation and circulation good.
10.20 a.m. Cannula in vein of fore-leg connected with burette containing Ringer's solution.
10.25 a.m. Injection begun. Brain as before.
10.33 a.m. 25 cc. in. Brain as before.
10.38 a.m. 50 cc. in. Brain slightly more convex.
10.45 a.m. 90 cc. in. Brain as before.
10.50 a.m. 100 cc. in. Brain normally convex, shows no bulging. Pulsation free, circulation good. Injection stopped.
11.10 a.m. Brain lies normal as before. Pulsation free.
11.26 a.m. Brain lies normal as before. Ether to death. Immediately injected with 10 per cent formaldehyde until vessels well filled, then head cut off and immersed in same solution. Original relations fairly well preserved after fixation.

With the control afforded by these experiments in which the skull was opened and in which there was no intravenous injection, or else the introduction of Ringer's solution, an interpretation of the results of the intravenous injection of hypotonic and hypertonic solutions may be safely attempted.

Water. A number of observations on cats with single and bilateral openings of the skull have been made, during and following the intravenous injection of sterile distilled water which, as noted in a previous section of this paper, has been shown to bring about an increase in the volume of the brain in the unopened skull. The conditions prevailing in these experiments were similar to those maintained during the injection of Ringer's solution. In all these animals the brain, which lay normally convex in the trephine openings, began to protrude very soon after the intravenous injection of distilled water was started. This bulging increased throughout the period of injection and reached its maximum usually in from ten to twenty minutes after the completion of the introduction of water. Tense herniae of the brain through the trephine openings were thus produced. The tension was in all cases so great that cerebral pulsation ceased before the swelling reached its maximum. The pressure of the brain on the dura at the edges of the trephine openings was usually marked enough to stop the circulation in

the dural flaps. These triangular flaps were stretched, pulled and rolled back into the interval between the hernia and the cut edge of the trephine opening. Figure 9 shows the result of such an experiment and presents fairly well the appearance of the cerebral hernia before the sacrifice of the animal, although fixation in formaldehyde at death of the animal caused some subsidence of the hernia. When observed just before the death of the cat, usually thirty minutes after the completion of the injection of water, the brain protruded, in most of our experiments, at least 4 mm. beyond the outer table of the skull; in one animal which gave a very marked reaction, the height of the hernia at the end of the observation was 8 mm.

The protocol of an experiment in which there was a bilateral opening of the skull and the intravenous injection of 100 cc. of sterile distilled water, is given below:

No. 1536. Adult female cat. Weight 2,430 grams. Intravenous water

3.50 p.m. Ether with intratracheal tube.
4.00 p.m. Double subtemporal decompression.
4.15 p.m. Dura opened. Brain lies with normal convexity, pulsating freely. No trauma to brain or membranes.
4.18 p.m. Cannula in vein of fore-leg connected with burette containing sterile distilled water.
4.19 p.m. Injection begun. Brain bulges immediately after beginning of injection.
4.25 p.m. Brain bulges more.
4.31 p.m. 50 cc. in. Brain bulges markedly with pulsation; circulation good.
4.35 p.m. 75 cc. in. Brain bulges markedly. Pulsation slight, circulation good.
4.45 p.m. 100 cc. in. Injection stopped. Brain in tense hernia. Pulsation slight on left side. No pulsation on right side.
5.00 p.m. Brain markedly herniated. No pulsation on either side.
5.15 p.m. Brain in tense hernia on both sides—stops circulation in dural flaps.
5.16 p.m. Ether to death. Immediately injected through the heart with 10 per cent formalin and when vessels filled, head cut off and immersed in same solution. After immersion hernia remains about as before. Convolutions slightly more rounded and whole hernia slightly flatter.

On section, these brains present the appearance seen in figure 12 (after single decompression) and in figure 15 (after bilateral opening of the skull). A considerable dislocation of brain substance is apparent with some flattening of the gyri and narrowing of the sulci; but the general impression gained from an examination of these sections is different from that resulting from observation of sections, as shown in

figures 5 and 7, of brains obtained from animals subjected to the intravenous injection of water, but with unopened skulls. In the sections of the brains which were allowed to herniate, the impression of extreme tenseness and turgidity is not outstanding as is the case with the brains which were restrained by unopened dura and skull. The difference is undoubtedly to be explained by the different mechanical limitations to expansion present in the two types of experiment.

It is apparent from the foregoing that, under experimental conditions similar to those prevailing in the control observations on brains with opened skull (with or without the intravenous injection of Ringer's solution), the intravenous introduction of water causes a marked herniation of cerebral substance resulting from the increase in the volume of the brain. It is also worthy of note that the gross appearance of sections of such brains is different from that previously observed in sections of brains of animals receiving water intravenously but with unopened skulls.

Salt. Intravenous injections of a hypertonic solution (30 per cent sodium chloride), which produced a marked reduction in volume of the brain in animals with unopened skulls, have been given to a number of cats in which openings in the skull and dura were made on one or both sides. The conditions prevailing in these experiments were similar to those already described for the control observations. Following the intravenous injection of 30 per cent sodium chloride, the normal convexity of the brain in the trephine opening disappears soon after the injection is begun, so that the brain is seen to lie flat. As the intravenous injection of the salt is continued, the brain falls away from the skull until the surface presented becomes concave. The maximum shrinkage has been observed usually in from fifteen to thirty minutes after the completion of the injection, when the brain lies flaccid, 3 to 4 mm. below the inner table of the skull, with only very slight visible pulsation. In figure 11 is shown the result of an experiment in which a single opening was made in the skull, and 16½ cc. of a solution of 30 per cent sodium chloride injected intravenously. The photograph reproduced in this figure was taken after the fixation of the head in 10 per cent formalin and does not show the marked shrinkage which was so striking at the end of the experiment. As has been emphasized, the methods of fixation employed do not preserve, with the accuracy desired, the relations existing during life; in spite of this difficulty it is readily apparent, from figure 11, that here the skull is only partially filled by this markedly shrunken brain.

The individual reaction and tolerance of cats to intravenous injections of a hypertonic solution of sodium chloride and the quantities most effective in producing a decrease in the volume of the brain, have been discussed in a preceding section of this paper. In these observations with the opened skull, doses approaching the limit of the animal's tolerance (16 to 20 cc.) have been administered; the same differences in individual reaction and tolerance, noted before, have been observed.

There is given below the protocol of a typical experiment in this series, in which there was a bilateral opening of the skull and dura and an intravenous injection of 20 cc. of a hypertonic solution of sodium chloride:

No. 1535. Adult female cat. Weight 2250 grams. Intravenous NaCl.

9.35 a.m. Ether with intratracheal tube.
10.10 a.m. Double subtemporal decompression.
10.15 a.m. Dura opened. Brain lies with normal convexity, pulsating freely. No injury to brain or membranes.
10.20 a.m. Cannula put in vein of fore-leg and connected with burette containing 30 per cent NaCl (Squibb).
10.23 a.m. Injection begun. Convexity of brain normal.
10.30 a.m. Brain as before. No hernia.
10.35 a.m. 11 cc. in. Both sides of brain receding—lie flat.
10.40 a.m. 14 cc. in. Brain fallen more—lies concave.
10.45 a.m. 16 cc. in. Brain fallen more.
10.50 a.m. 19 cc. in. Brain fallen still more.
10.54 a.m. 20 cc. in. Injection stopped. Brain markedly fallen. Circulation good, pulsation slight.
11.05 a.m. Brain markedly shrunk. Pulsation slight. Animal in good shape.
11.25 a.m. Brain far receded, pulsation slight. Brain lies 3 to 4 mm. away from the inner table of the skull.
11.26 a.m. Ether to death. Immediately injected through the heart with 10 per cent formaldehyde plus 1.5 per cent NaCl with cat lying on belly. Head cut off when vessels well filled and immersed in same solution. Within a few minutes after immersion brain rose in skull almost level with trephine opening.

After fixation in formalin and section, these brains, taken from animals with opened skulls and with intravenous injection of 30 per cent sodium chloride, are characterized by the condition shown in figure 14 (single skull opening) and figure 17 (bilateral subtemporal decompression). That there has been a decided decrease in the volume of the brain in both these cases is quite evident. Figures 12, 13 and 14 show sections of a series of brains taken from animals in which there was a single subtemporal decompression. In one animal (fig. 12) water was

injected intravenously; in another (fig. 14) 30 per cent sodium chloride, while in the third, (fig. 13) no injection was given. Figures 15, 16 and 17 show sections of brains from another series of animals in which bilateral openings were made in the skull and dura. Intravenous water was given in one animal (fig. 15), intravenous 30 per cent sodium chloride in another (fig. 17), but in the control (fig. 16) nothing was injected. A glance at these figures is quite sufficient to show that in the animal receiving the intravenous injection of a solution of hypertonic sodium chloride in both series, the brain has been markedly decreased in volume. A close examination of figures 14 and 17, and a comparison of these figures with figures 6 and 8, which resulted from experiments in which hypertonic solutions were injected intravenously but with the skull unopened, is extremely interesting in that, while there is evident shrinkage in both types of experiment, the way in which the brain was affected is different in the two. From figures 14 and 17 (opened skull) one gets an impression of marked compactness of the brain as a whole; this phenomenon is not so apparent in figures 6 and 8 (unopened skull). The well rounded gyri and the clearly apparent sulci, previously described as characteristic (following intravenous injections of hypertonic solutions) of brains arising from experiments in which the skull was unopened, are not to be found in brains after similar experimental procedures but with the skull opened. It is evident, then, that with the operation of the same factor which tends to produce a decrease in the volume of the brain, the form of the end result in the two cases is altered by the mechanical conditions imposed by the opened or unopened skull. It is thus apparent that when the brain is allowed by an opened skull to shrink and contract freely, the appearance of a greater decrease in total volume is obtained than in experiments where the force producing the reduction in volume must, as it were, pull against a partial vacuum furnished by the intact skull.

These observations make it clear that in brains unrestrained by the physical limitation of the closed cranium there is a marked decrease in volume after intravenous injection of hypertonic solutions of sodium chloride; but the resulting picture is different from that described for brains shrunken, after similar injections, within an intact skull.

In the course of the above experiments the failure of the brain of a very old cat to show marked swelling after the intravenous injection of water led to several observations on decidedly old animals. Two very old cats, following double temporal decompression, were given intravenous injections of 100 cc. of water. The brain of neither cat her-

niated markedly from increase in bulk. Another old cat, after double temporal decompression, was given an intravenous injection of 20 cc. of 30 per cent sodium chloride. The brain shrank away from the skull to a considerable extent, although not so far as is usually the case in younger individuals. It has been frequently noted that when exposed to view by operative procedure, the brains of these old cats look different from those of younger individuals. While they pulsate freely in the decompression openings, they lie flat and do not show so much convexity as is characteristic of the brains of younger individuals. The sulci of these brains of old cats seem wider and the gyri more rounded than those characteristic of younger cats. That the brains of these old animals react less readily to intravenous injections by changes in bulk than the brains of younger individuals seems certain from these observations; that increase in volume should be more difficult than decrease seems reasonable, in view of certain mechanical and other conditions existing within the cranium in old individuals.

Two very young cats weighing 1,300 and 1,500 grams, were selected as typical young adults, in which changes in brain bulk should be outspoken. Following double subtemporal decompression, one received an intravenous injection of 100 cc. of water, the other 16 cc. of 30 per cent NaCl. Both showed very well marked reactions, developing relatively as great swelling or shrinkage as has been seen in any animal.

HISTOLOGICAL EXAMINATION

The pronounced swelling, which occurs in the cat's brain after the intravenous injection of water, and the marked shrinkage which follows the intravenous injection of strongly hypertonic solutions, has led to the desire to correlate, if possible, these gross alterations with histological changes in the cerebral substance.

Mention has already been made in this paper of the fact that fixation with formaldehyde does not preserve accurately the gross form and size relations of the brain as seen prior to death of the animal, so that the preliminary observations here recorded may represent only very roughly the histological picture accompanying the various modifications of brain bulk. That there are marked histological changes is readily seen but their exact interpretation, particularly in regard to the representation of conditions prevailing prior to fixation, is a matter requiring further study. It is hoped that when a method of fixation is devised which will preserve the form and size relations accurately, more intelli-

gent histological and cytological observations may be made. The present findings are reported tentatively, pending an attempt to control the artifacts probably introduced by the technical methods employed.

The material available for this study was that resulting from the experiments described in the preceding parts of this paper. This material was preserved in formaldehyde, which was in most cases injected through the aorta immediately after the death of the animal. One series of brains fixed by Formaline-Zenker's fluid was used in an attempt to control the material preserved in formaldehyde. Sections were cut in paraffin, 10 μ thick; stained with haematoxylin and eosin, toluidine blue and fuchsin S, and with Mallory's and Van Gieson's connective tissue stains. All sections were made from blocks of cortex including the sulcus lateralis and parts of the two adjacent gyri, taken from the dorsal surface of the brain in the same transverse plane as the optic chiasma. In the animals where the skull was opened during the experiment, this block of cortex was found to be in the upper part of the trephine opening. Examination of sections of cortex taken from animals used in this study quickly showed that, when judged by the histological changes which accompany or follow gross shrinkage or swelling, these animals may be divided into two groups; those in which the skull and dura were opened during the experiment and those in which the cerebral cavity remained intact. The histological changes seen in sections of non-decompressed brains following various intravenous injections are quite marked and constant in the material studied, but sections from decompressed brains fail to exhibit the same marked differences from the control. It is quite evident, then, that following decompression the brain may adjust its volume; it may herniate, being only partially restricted by the dura, or collapse freely. This comparative freedom to contract or expand probably explains the finding of similar histological pictures in the normal decompressed controls, and in the decompressed brains following intravenous injection of water or salts. All the specimens tended to approach the normal when the brains were allowed to contract or expand with freedom, but when the alterations in cerebral volume were limited by the closed cranium, the factors responsible for the macroscopic changes in brain bulk produced also marked microscopic changes. Although these histological changes as observed have doubtless been altered by the technical methods employed in the preservation of these brains, their constancy is ample evidence that they are indicative of certain fundamental changes in the brain substance, even though they may represent very

inaccurately the actual conditions prevailing prior to death. The technical procedures employed have been practically uniform for all brains, so that the artifacts produced may be considered as constant.

Skull unopened

Normal. Control sections were made from blocks of cortex taken from animals receiving no intravenous injection and from animals in which Ringer's solution was introduced intravenously. While it is apparent that there are artifacts in these sections, due probably to the method of fixation, they furnish a reasonable control for sections of brains taken from animals receiving intravenous injections of various concentrations. An examination of sections made from brains of animals in which Ringer's solution was introduced intravenously and a comparison of these sections with normal controls shows no fundamental differences in the two, so far as our observations go. These sections, then, furnish a reasonable standard with which the sections from animals subjected to other experimental procedures may be compared.

Water. The general appearance of sections of the cortex made from non-decompressed brains following the intravenous injection of water, is that of a swollen tissue. The sulci are quite narrow and the gyri tend to be flat. The smaller vessels seem, if different from normal, contracted within normal or slightly expanded perivascular spaces. The intercellular material of the gray matter seems inflated, the spaces found among the interlacing cell processes appearing larger than in control sections. Many of the large dendrites which rise perpendicular to the free surface of the gyrus are apparently larger than in the normal. Under higher magnifications the nuclei of nerve cells seem compact, the whole nucleus being perhaps slightly contracted. About the cells in most of the water brains examined, there is an evidently enlarged pericellular space, the general impression being that the cell itself is contracted away from the surrounding tissue. The occurrence of enlarged pericellular spaces in the gray matter of the cortex, following the intravenous injection of water, is most striking and constant. Pericellular spaces are apparent in sections of normal cortex, particularly about the larger cells, but following the introduction of water intravenously these spaces, even about the smaller cells, are evidently considerably enlarged. In this material the enlarged pericellular spaces and other histological evidences of change may be interpreted as due

to conditions within the brain, different from those existing normally, and produced by the intravenous injection of a hypotonic solution.

Salt. Sections of the cortex taken from brains following intravenous injection of 30 per cent sodium chloride, under low magnification, show in general an appearance of tissue contraction, the sulci being wide and the substance of the brain condensed. In practically all of the salt brains examined, the cortical capillaries seem distended. This distension may be explained partially, perhaps, by the arterial injection with formaldehyde (a procedure carried out with the normal and water brains) but it is a constant finding in the salt brains and may be taken as an indication of some constant factor in the fluid concentration within the nervous tissue. In most of the salt brains the perivascular spaces are apparent but not enlarged. The intercellular felt-work in the gray matter seems compacted and denser than in normal sections. The outstanding feature of the histological picture presented by these sections of salt brains concerns the occurrence of a marked clear space about the nuclei of many cells. These clear spaces vary in size from a slight space about a well-rounded nucleus to a wide space around a markedly crenated nucleus. These spaces increase in size in the gray matter from the medullary core out to the surface layers of each gyrus. The nuclei appear condensed, the chromatin being aggregated. The Nissl substance in the cytoplasm is at the periphery of the cell, the perinuclear spaces being between the nuclear membrane and a marginal ring of Nissl substance. In sections stained with toluidine blue and fuchsin S, these spaces are seen to be partially filled with a fluid-coagulum which stains with fuchsin. This coagulum in most cases has shrunk away from the nucleus toward and against the ring of Nissl substance. No pericellular spaces are usually apparent about the cells showing such perinuclear spaces. The pericellular spaces may be observed occasionally however in the deeper layers of the gray matter about the larger cells. As noted above, the perinuclear spaces are more marked and the nuclei more crenated in cells of the surface layers of the cortex. It seems evident, then, after a comparison of these sections with normal control sections, that in these brains the intracellular perinuclear spaces and other histological evidences of change in the brain substance may be attributed to changes brought about by the intravenous injection of a hypertonic solution.

Skull opened

Examination of sections of brains following intravenous injections of solutions of various concentration in animals with opened skulls reveals a histological picture quite similar in all. There are, of course, minor differences in the sections examined, but such differences do not seem to be due to any changes brought about by the intravenous injection. The decompression has evidently allowed each brain to expand or contract freely and to adjust its fluid distribution so that no essential histological differences are noticeable, all retaining very nearly a normal appearance. That there may be in these brains fundamental histological and cytological differences not revealed by the methods employed, is probable, but further work is necessary to establish such differences. With the methods employed it is certain that the decompressed brains do not show the histological characteristics which are so evident in the non-decompressed brains.

An exact interpretation of the above observations on the histological changes in the cortex of the brain following intravenous injections of hypotonic and hypertonic solutions can not now with reason be attempted. That there are histological changes in brains unrelieved by decompression is certain, but these changes need more accurate study and control before any sane effort can be made to explain them accurately. The changes described have no doubt been influenced by the technical methods employed; these methods may have, in addition, masked or destroyed other histological evidences of changes produced by the intravenous injections. Until one finds a method of fixation which will preserve the form and size relations of the brain accurately and at the same time will make possible accurate histological study, the above observations may be accepted tentatively as an indication that changes in the brain substance, recognizable histologically, do occur following intravenous injections of solutions of various concentrations.

DISCUSSION OF RESULTS

The experimental alteration of the volume of the brain by intravenous injections of hypotonic and hypertonic solutions has not, so far as we have been able to find, been previously recorded in the literature. The ease and rapidity of these changes in brain volume are of considerable interest in view of the old idea of the incompressible character of the brain and its relation to the conception of a constant vascular volume within the cranial cavity.

The hypothesis that the volume of the blood circulating within the cranium must at all times be constant was first brought forward by Alexander Monro, the younger (2) in 1783. At this time he wrote that

> as the substance of the brain, like that of other solids of our body, is nearly incompressible, the quantity of blood within the head must be the same at all times, whether in health or disease, in life or after death, those cases only excepted in which water or other matter is effused or secreted from the blood-vessels; for in these cases, a quantity of blood equal in bulk to the effused matter, will be pressed out of the cranium.

This viewpoint advanced by Monro was accepted and elaborated by Kellie (3) in 1824, who based his ideas upon observations on men frozen to death and upon experiments on animals. His conclusions were that a state of bloodlessness did not exist in the brains of animals killed by bleeding, that the quantity of blood in the cerebral vessels was not affected by posture or gravitation, that congestion of these vessels was not found in those conditions in which it might be well expected (hanging, etc.) and that compensatory readjustments between the different sets of cerebral vessels always maintained a constant vascular volume. Subsequently Kellie wrote

> that in the ordinary state of these parts we can not lessen, to any extent, the quantity of blood within the cranium, by arteriotomy or venesection; whereas if the skull of an animal be trephined then hemorrhage will leave very little blood in the brain.

Within the next two decades following the publication of the results of Kellie's experiments, many clinical observations were reported in substantiation of this conception—that the vascular content of the brain was at all times practically constant. This Monro-Kellie doctrine received wide publicity through its acceptance by Abercrombie (4). This eminent surgeon, in discussing apoplexy, thus summed up his views on the subject (p. 300):

> In this investigation it is unnecessary to introduce the question, whether the brain is compressible, because we may safely assert that it is not compressible by any such force as may be conveyed to it from the heart through the carotid and vertebral arteries. Upon the whole, then, I think we may assume the position as being in the highest degree probable, that, in the ordinary state of the parts, no material change can take place in the absolute quantity of blood circulating in the vessels of the brain.

Burrows in 1843 (5) was probably the first to question the absolute accuracy of this doctrine which so firmly considered the brain as of

fixed incompressible bulk. He emphasized strongly the importance of the cerebro-spinal fluid, as the means of replacing the loss of blood during hemorrhage, for he felt that the amount of intracranial blood was obviously diminished by systemic bleeding.

"Whether the vacated space is replaced by serum, or resiliency of the cerebral substance under diminished pressure, is another question" was Burrows' summary of the possible readjustment for variations in the volume of the cerebral blood. As far as can be ascertained, this is the first statement of the view that the volume of the brain may be altered in accord with pathological or physiological conditions within the cranium. Burrows presents one of the most satisfactory conceptions of the whole process of fluid changes within the cranium (p. 32):

> Those who have maintained this doctrine of the constant quantity of blood within the cranium, have not, I believe, taken into due consideration that large proportion of the contents of the cranium which consists of extra-vascular serum. Regarding this serum as an important element of the contents of the cranium, I admit that the whole contents of the cranium, that is, the brain, the blood, and this serum together, must be at all times nearly a constant quantity.

It was only when the subject of a constant blood volume in the cranium was subjected to experimental test that reliable data were obtained. Kussmaul and Tenner (6) demonstrated the unreliability of post-mortem observations and came to the conclusion, advanced by Burrows and supported by the experimental work of Donders (7), that variations in the total volume of blood in the cranium occurred. These early experiments, as pointed out by Leonard Hill (8) in 1896, are not conclusive as variation in the blood volume in one part of the cerebral vascular system might well be compensated by readjustments in another. Following many other investigators who used various methods of experimental attack, Leonard Hill concluded that (p. 77): "The volume of blood in the brain is in all physiological conditions but slightly variable."

More recently (1914) Dixon and Halliburton (9), in the course of an extensive study of the cerebro-spinal fluid, have come to the conclusion "that the cranial contents cannot any longer be regarded as a fixed quantity without the power of expanding or contracting in volume."

It must be assumed, however, that with certain reservations, the data favor the idea of a relatively fixed total volume of the cranial contents but with the capacity for change in any one of the three chief elements concerned.

The conception of a more or less constant cranial content is closely related to the questions which are naturally called forth by the experimental modification of brain bulk, detailed in foregoing sections of this paper. For within the closed cavity the alteration in volume of any element must be at the expense of the other elements. First of the possible explanations of the experimental alteration in brain bulk is that relating to the blood volume of the cranium. Are the vascular readjustments following the intravenous injection of hypotonic and hypertonic solutions sufficient to account for the definite change in brain bulk? In one of his original experiments, in which the cranium of a dog had been trephined, Kellie observed a recession of the brain away from the skull during exsanguination. Ecker (9) also observed in a trephined animal a remarkable shrinkage of the brain when the loss of blood from division of the carotids became excessive. The converse of this vascular diminution of the brain volume was recorded also by Ecker, who found that pressure on the thorax of a trephined dog caused protrusion of the brain into the cranial opening. Burrows also comments on this possibility of hernia through the trephine opening occurring in those cases in which the blood supply to the brain was markedly increased. These early observations on the relation of the cranial vascular supply to the volume of the brain during life have been confirmed and substantiated by many other workers on the cerebral circulation and cerebro-spinal (intracranial) pressure.

In our own experiments the modification of brain bulk has been produced both in the opened and in the intact skull. Observations on venous and arterial pressures under such experimental conditions have been made; these will be reported at another time. But that these alterations in brain bulk are independent of changes in volume of the blood in the vascular bed, may be deduced from other findings. The fact that similar changes occur in both the trephined and the unopened skull is strong evidence against the view that these changes in brain bulk depend on alterations in vascular volume and the persistence of the anatomical change after formalin fixation makes such a view untenable. For it must be assumed that with death of the animal, opening of the chest, introduction of a cannula in the aorta, injection of formalin through this vessel and release of the pressure by incising the right auricle, any vascular alterations existing in life are no longer maintained; so that the persistence, after such fixation, of a given brain bulk, if due simply to the amount of blood in the capillary bed and other vessels of the brain, would be impossible. That changes in

bulk do persist after fixation as above, is ample evidence that such changes are maintained by some fundamental and comparatively stable alterations in the substance of the brain itself. A further fact, which shows that the changes in volume of the brain as produced in these experiments are fundamentally independent of vascular alterations, is that brains fixed by immersion in formalin, as well as those preserved by arterial injection of the fixing agent, retain in part the changes in bulk produced by intravenous injection. Following several experiments in which, after opening of the skull the brain bulk was changed by intravenous injection, the heads of the animals were cut from the body and immersed in formalin. That the skull and dura were freely open during experimentation in these cases and that all the vessels of the neck were severed before fixation by immersion and not by injection and that after such treatment the brain still maintained the volume change brought about by the intravenous injection, are further evidence that the changes in bulk are independent of vascular alterations. While, as has been emphasized, the method of preservation does not accurately maintain the condition existing in life, it nevertheless makes possible the recognition of undeniable evidence of change in brain bulk. Vascular alterations may account for some changes in brain bulk which occur in the living animal, but the changes persisting in death and after the technical procedures employed in this investigation are quite evidently due to some other cause.

The other variable factor which may operate within the cranium in producing changes in brain bulk involves the cerebro-spinal fluid. We have already noted the lasting rise in the pressure of the cerebro-spinal fluid following the intravenous injection of a hypotonic solution, and the production of swollen brains by such injections. Conversely, a definite decrease in the size of the brain has been found in those cases in which the pressure of the cerebro-spinal fluid has been markedly lowered by intravenous injection of hypertonic solutions. In considering these results it becomes rather difficult to determine with absolute accuracy the primary factor involved in producing these alterations. Does the modification of the bulk of the brain determine the pressure-change in the cerebro-spinal fluid or are both dependent individually on some more fundamental cause? That change in brain volume in our experiments is not caused alone by changes in the pressure of the cerebro-spinal fluid is demonstrated by its occurrence in the opened skull, for with the trephine opening and the dura incised, the fluid pressure becomes minimal, and any rise in pressure is within certain limits

relieved. That fundamental osmotic changes in the blood are responsible for the changes in the pressure of the cerebro-spinal fluid, following intravenous injections of solutions of various tonicity, seems a reasonable conclusion. Although it is probable that change in the volume of the brain may affect the pressure of the cerebro-spinal fluid, and possible that changes in the pressure of the fluid may alter the bulk of the brain, in these experiments there is evidence that primary alterations in brain bulk and cerebro-spinal fluid pressure, both, are caused by fundamental osmotic changes in the blood supplied to the brain.

Such considerations force one to conclude that the alteration in the volume of the brain following intravenous injections of hypotonic or hypertonic solutions is quite independent of change in either the volume of the blood supply to the brain or of the pressure of the cerebro-spinal fluid. With the diminution in bulk the pressure of the cerebro-spinal fluid falls—a partial compensation for the evacuated space formerly occupied by the brain of normal size. Conversely, an increased bulk of the brain may cause dislocation of a certain volume of the cerebro-spinal fluid, thus raising its pressure as determined in a manometer.

Relating this experimental modification of the brain bulk to the restricted Monro-Kellie doctrine it becomes evident that another variable factor must be introduced. The brain should no longer be considered as incompressible and of fixed volume as the early writers assumed it to be, but as subject to variation in size under experimental conditions. Monro, of course, qualified his theory by consideration of matter "effused or secreted from the blood vessels," and Burrows suggested that the brain possessed "resiliency of the cerebral substance under diminished pressure." Pathologically the increase in bulk of the brain is well known in the cerebral edemas of trauma, acute infections and certain other conditions. Similarly, pathological states characterized by diminished volume of the brain are also quite common. The Monro-Kellie doctrine then requires marked modification; the view so well advanced by Burrows is probably the more correct. This leads one to assume that the cranial cavity is relatively fixed in volume and is completely filled by brain, cerebro-spinal fluid and blood; variations in any one of the three elements may occur, compensation being afforded by alteration in the volume of one or both of the remaining elements.

The underlying processes involved in the modification of brain bulk by the intravenous injection of hypertonic and hypotonic solutions seem concerned then with osmotic changes in the blood. That the osmotic pressure of the blood is an essential factor in such experimental

changes in brain bulk is shown by the fact that no alteration in the volume of the brain follows relatively large doses of Ringer's solution (100 cc. in a cat) but occurs promptly on intravenous injection of far smaller amounts of distilled water or concentrated salines. Just how this change in osmotic value of the blood affects the brain tissue and alters its volume can only be speculated upon at the present time. The change is limited apparently by the potential distensibility or contractility of the brain in the particular animal used. Thus, in old cats, the alterations in brain volume have not been so marked as in younger animals, though the contractility seems to persist longer than the distensibility. On the other hand, in young animals the change in cerebral volume is of far easier accomplishment. Capacity for osmotic changes in these animals must be about the same; the resultant modification of the brain bulk then is limited by anatomical factors. Finally, in the closed skull certain changes take place, limited by the potential powers of change in the brain itself and by the intradural capacity; in the trephined skull, the only limitation to change is the intrinsic capability for contraction or expansion of the brain itself.

SUMMARY

1. Intravenous injection of a hypertonic solution (30 per cent NaCl or saturated $NaHCO_3$) is followed by a marked decrease in size of the brain; when the skull is opened the brain may be seen to fall away several millimeters from the inner surface of the skull after such injection.

2. Intravenous injection of a hypotonic solution (water) causes a marked swelling of the brain; when openings are made in the skull the brain will rise, forming tense herniae protruding several millimeters through the trephine openings.

3. These changes are independent of the volume of the fluid injected and are probably due to fundamental osmotic effects of the hypotonic and hypertonic solutions.

4. The brains of old cats fail to respond readily to intravenous injection, particularly to the intravenous injection of hypotonic solutions.

5. Internal changes, recognizable histologically, have been found quite constantly in the brains of animals which have been given intravenous injections of hypertonic or hypotonic solutions and which have not been trephined. On the contrary, in animals in which the skull is opened and the brain thus allowed to change its volume freely, these histological changes have not been demonstrated.

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PLATE I

Fig. 1. Photograph × ¾. Cat no. 1304. Adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 75 minutes, with intravenous injection of 35 cc. sterile distilled water. Pressure rose from 105 mm. to 175 mm. following injection. Sacrificed with ether 30 minutes after completion of injection. Fixed by injection with 10 per cent formalin. Dura not removed.

Fig. 2. Photograph × ¾. Cat no. 1371. Adult male. Intravenous injection of 20 cc. 30 per cent NaCl (Squibb) in 5 cc. doses given at 30 minute intervals. Sacrificed with ether 2 hours after first and 30 minutes after last dose. Fixed by injection with 10 per cent formalin. Dura not removed.

Fig. 3. Photograph × 1. Cat no. 1402. Adult female. Control. Well-nourished, normal animal. Sacrificed with ether. Transverse section through optic chiasma. Fixed by injection with 10 per cent formalin.

Fig. 4. Photograph × 1. Cat no. 1309. Young adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 81 minutes, with intravenous injection of 12 cc. Ringer's solution. Initial pressure 90 mm., final pressure 110 mm. Sacrificed with ether 1 hour after completion of injection. Transverse section through optic chiasma. Fixed by injection with 10 per cent formalin.

Fig. 5. Photograph × 1. Cat no. 1304. Transverse section through optic chiasma of brain shown in figure 1.

Fig. 6. Photograph × 1. Cat no. 1371. Transverse section through optic chiasma of brain shown in figure 2.

Fig. 7. Photograph × 1. Cat no. 1303. Adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 84 minutes, with intravenous injection of 20 cc. sterile distilled water. Pressure rose from 106 mm. to 195 mm. Sacrificed with ether 1 hour after completion of injection. Transverse section through optic chiasma. Fixed by injection with 10 per cent formalin.

Fig. 8. Photograph × 1. Cat no. 1364. Young adult female. Occipito-atlantoid puncture. Continuous observation of pressure of cerebro-spinal fluid for 90 minutes, with intravenous injection of 10 cc. saturated aqueous solution of sodium bicarbonate. Pressure fell from 115 mm. to below zero. Sacrificed with ether 70 minutes after completion of injection. Transverse section through optic chiasma. Fixed by injection with 10 per cent formaldehyde.

PLATE II

Fig. 9. Photograph × ¾. Cat no. 1524. Adult female. Weight 2,350 grams. Temporal decompression on left side. Intravenous injection of 100 cc. sterile distilled water. Marked hernia of brain through trephine opening 3 mm. beyond outer table of skull beginning with the injection and persisting until animal was sacrificed with ether 35 minutes after completion of injection. Fixed by immersion with 10 per cent formalin. Trephine opening made on right side after the beginning of fixation.

Fig. 10. Photograph × ¾. Cat no. 1531. Adult male. Weight, 2,330 grams. Temporal decompression on left side. Intravenous injection of 100 cc. Ringer's solution. Brain lies convex with no hernia or evidence of swelling or shrinkage throughout the experiment. Sacrificed with ether 36 minutes after completion of injection. Fixed by immersion with 10 per cent formalin. Trephine opening made on right side 45 minutes after the beginning of fixation.

Fig. 11. Photograph × ¾. Cat no. 1506. Adult male. Weight, 2,100 grams. Temporal decompression on left side. Intravenous injection of 16½ cc. 30 per cent NaCl (Squibb). Brain rises slightly with beginning of injection but falls away from skull before injection is finished. Thirty minutes later lies concave, 3 mm. below inner table of skull at forward edge of opening. Sacrificed with ether 33 minutes after completion of injection. Fixed by immersion with 10 per cent formalin. Trephine opening made on right side after the beginning of fixation.

Fig. 12. Photograph × 1. Cat no. 1501. Adult female. Weight 2,400 grams. Temporal decompression on left side. Intravenous injection of 100 cc. sterile distilled water. Brain rises as injection begun; hernia increases during injection; brain bulges markedly through trephine opening with protrusion in center about 8 mm. beyond outer table of skull, 35 minutes after injection finished. Sacrificed with ether 37 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section just behind optic chiasma. Dura removed.

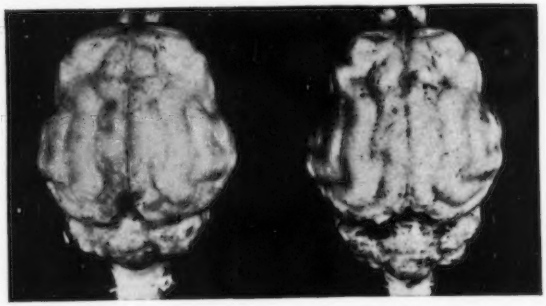
Fig. 13. Photograph × 1. Cat no. 1503. Adult female. Weight 2,260 grams. Control. Temporal decompression on left side. No intravenous injection. Brain lies convex about level with the outer table of the skull throughout experiment. Sacrificed with ether 35 minutes after completion of decompression. Fixed by injection through aorta with 10 per cent formalin. Transverse section just behind optic chiasma. Dura removed.

Fig. 14. Photograph × 1. Cat no. 1505. Adult female. Weight 2,050 grams. Temporal decompression on left side. Intravenous injection of 16½ cc. 30 per cent NaCl (Squibb). Brain bulges markedly during injection, but immediately begins to subside on completion of injection until in 30 minutes it lies concave 1 mm. below inner table of skull. Sacrificed with ether 36 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section just behind optic chiasma. Dura removed.

Fig. 15. Photograph × 1. Cat no. 1536. Adult female. Weight 2,430 grams. Double temporal decompression. Intravenous injection of 100 cc. sterile distilled water. Beginning with the injection brain rises in a tense hernia on both sides. Sacrificed with ether 31 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section through optic chiasma. Dura removed.

Fig. 16. Photograph × 1. Cat no. 1532. Adult male. Weight 2,250 grams. Control. Double temporal decompression. No intravenous injection. Throughout experiment brain lies convex with no bulging. Sacrificed with ether 20 minutes after completion of decompression. Fixed by injection through aorta with 10 per cent formalin. Transverse section through optic chiasma. Dura removed.

Fig. 17. Photograph × 1. Cat no. 1541. Young male. Weight 1,500 grams. Double temporal decompression. Intravenous injection of 16 cc. 30 per cent NaCl (Squibb). Beginning with injection brain falls away from skull lying about 3 mm. below inner table 20 minutes after injection stopped. Sacrificed with ether 55 minutes after completion of injection. Fixed by injection through aorta with 10 per cent formalin. Transverse section through optic chiasma. Dura removed.



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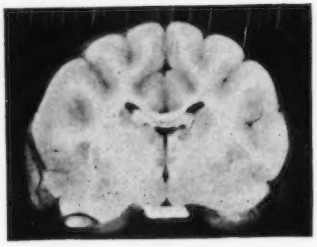

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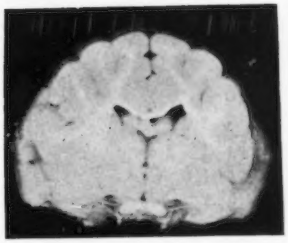

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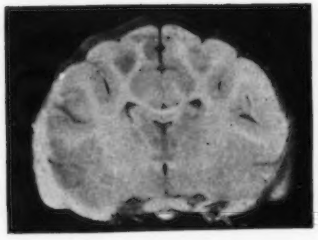

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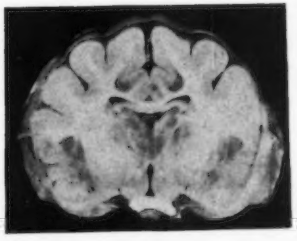

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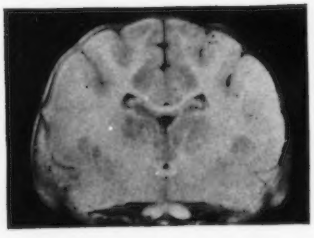

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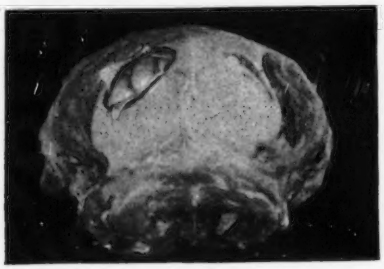
(Weed and McKibben: Experimental alteration of brain bulk)

9



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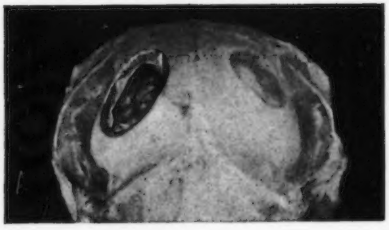

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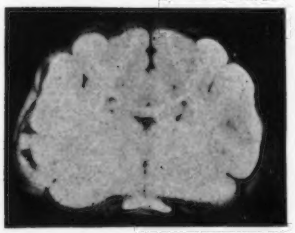

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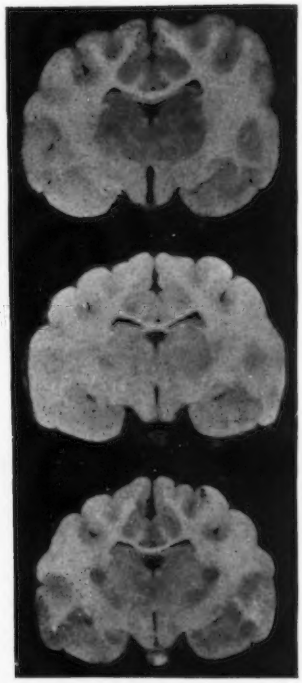

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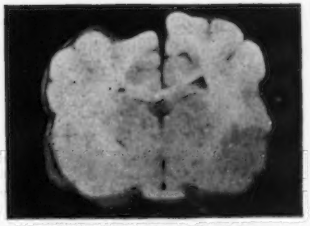

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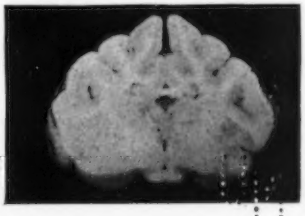

17

(Weed and McKibben: Experimental alteration of brain bulk)

Unusual Interest Has Been Shown in the Literature We Are Circulating

Regarding:

Ovarian Functionation
(Corpus Luteum)

Intramuscular Injections
(Mercury Salicylate)
(Arsphenamine)

Blood Conditions
(Acidosis.—Urea Content)

Diagnostic Conveniences
(Blood Collecting Devices)

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